The synthesis of ligands at the D-myo-inositol 1,4,5-trisphosphate receptor based on adenophostin A

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The synthesis of ligands at the D-*myo*-inositol 1,4,5-trisphosphate receptor based on adenophostin A

Submitted by Rachel D. Marwood
for the degree of PhD of the University of Bath

Signed... Rachel D. Marwood.....

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Abstract

The synthesis of adenophostin A, a recently discovered exceptionally potent agonist at the 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] receptor, and a series of adenophostin A analogues is described. The key adenosine intermediate, 5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine was made *via* a route involving selective N^6 dimethoxytritylation in the presence of a free 5'-hydroxyl, and regioselective reduction of a 2',3'-*O*-*p*-methoxybenzylidene acetal. Glycosidation of this intermediate with a protected glucopyranoside phosphite donor followed by selective deprotection, phosphorylation and complete deprotection resulted in adenophostin A. This synthetic adenophostin A was found to be pharmacologically equipotent with a sample of natural adenophostin A. In addition a sample of our synthetic adenophostin A was examined by a collaborator using potentiometric and NMR titrations. The binding orientation of adenophostin A at the Ins(1,4,5)P_3 receptor was investigated by means of the synthesis and biological evaluation of two sugar modified analogues in which the glucose portion of adenophostin A was replaced by mannose and xylose. 3-*O*-α-D-Mannopyranosyl adenosine 2',3",4"-trisphosphate was found to have greatly reduced Ca^{2+} releasing activity compared with adenophostin A, while 3'-*O*-α-D-xylopyranosyl adenosine 2',3",4"-trisphosphate was found to be almost equipotent with adenophostin A. Methyl 3-*O*-α-D-glucopyranosyl-β-D-ribofuranoside 2,3',4'-trisphosphate (ribophostin), in which the adenine ring of adenophostin A has been effectively deleted was synthesised in a convergent route from D-glucose and D-ribose, utilising a trichloroacetimidate glycosidation strategy. Ribophostin was found to be almost equipotent with Ins(1,4,5)P_3. Further reduction of the adenophostin A structure led to the design and synthesis of [(3S,4R)-3-hydroxytetrahydrofuran-4-yl] α-D-glucopyranoside 3,3',4-trisphosphate (furanophostin) an analogue in which the *O*-methyl and 4-hydroxymethyl moieties have been deleted. Like ribophostin, furanophostin was almost as active as Ins(1,4,5)P_3. To enable the synthesis of base modified adenophostin A analogues a versatile disaccharide intermediate, 1,2,3',4'-tetra-*O*-acetyl-2',5,6'-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose was synthesised. Vorbrüggen condensation of this intermediate with purine and imidazole led to the synthesis of 3'-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine 2',3",4"-trisphosphate (purinophostin) and 3'-*O*-α-D-glucopyranosyl)-1-β-D-ribofuranosidoimidazole 2',3",4"-trisphosphate (imidophostin) respectively. Purinophostin was equipotent with adenophostin A, while imidophostin exhibited activity similar to that of Ins(1,4,5)P_3. These data contribute to the construction of a general binding model for adenophostin A at the Ins(1,4,5)P_3 receptor.

Publications

Jenkins D J, Marwood R D, Potter B V L (1997) A disaccharide polyphosphate mimic of D-*myo*-inositol 1,4,5-trisphosphate. *Chem Commun*: 449–450

Marchant J S, Beecroft M D, Riley A M, Jenkins D J, Marwood R D, Taylor C W, Potter B V L (1997) Disaccharide polyphosphates based upon adenophostin A activate hepatic D-*myo*-inositol 1,4,5-trisphosphate receptors. *Biochemistry* **36**: 12780–12790

Marwood R D, Riley A M, Correa V, Taylor C W, Potter B V L (1999) Simplification of adenophostin A defines a minimal structure for potent glucopyranoside-based mimics of D-*myo*-inositol 1,4,5-trisphosphate. *Bioorg. Med. Chem. Lett.* **9**: 453–458

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Dedication

To my family, for their never ending love and support, and to Ian for being there when it mattered.

Abbreviations

$[\alpha]_D$	specific rotation at 589 nm
All	allyl
AMP	adenosine 5'-monophosphate
Ar	aryl
Bn	benzyl
bp	boiling point
Bz	benzoyl
cADPR	cyclic adenosine diphosphate ribose
cAMP	adenosine 3',5'-cyclic phosphate
cGMP	guanosine 3',5'-cyclic phosphate
CIF	calcium influx factor
°C	degrees Celcius
COSY	correlated spectroscopy
δ	chemical shift
d	doublet (spectral)
dd	doublet of doublets (spectral)
ddd	doublet of doublet of doublets (spectral)
DAG	1,2-diacylgylcerol
DCM	dichlormethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarisation transfer
DIBAL	diisobutylaluminium hydride
DMF	*N,N*-dimethylformamide
DMTr	dimethoxytrityl
DMSO	dimethylsulphoxide
EC_{50}	concentration producing 50% maximal response
ER	endoplasmic reticulum
FAB	fast atom bombardment
h	hour
HPLC	high-performance liquid chromatography
Hz	hertz
I_{CRAC}	calcium-release activated calcium current
Ins(1,4,5)P_3	1D-*myo*-inositol 1,4,5-trisphosphate
J	coupling constant (in NMR)
L	litre(s)
lit	literature (reference)
μ	micro
m	milli, multiplet (spectral)

M	moles per litre
MCPBA	*m*-chloroperoxybenzoic acid
MHz	megahertz
min	minute(s)
MIPP	multiple inositol polyphosphate phosphatase
mM	millimoles per litre
MMTr	monomethoxytrityl
mol	mole(s)
mp	melting point
MS	mass spectrometry
m/z	mass to charge ratio (mass spectrometry)
NAD	nicotinamide adenine dinucleotide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
PH	pleckstrin homology
PLC	phospholipase C
PMB	*p*-methoxybenzyl
ppm	parts per million (in NMR)
Ptd	phosphatidylinositol
q	quartet (spectral)
R_f	retention factor (in chromatography)
s	singlet (spectral)
t	triplet (spectral)
*p*TSA	*p*-toluenesulphonic acid
TEAB	triethylammonium bicarbonate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultra-violet

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Chapter one

Introduction

1 Introduction

1.1 Calcium signalling

The importance of Ca^{2+} in cellular function is reflected in the way in which levels of free intracellular Ca^{2+} in the cells of eukaryotic organisms are tightly controlled by a large number of coordinated regulatory mechanisms.[1-4] This regulation is crucial to many specialised functions of cells; for example changes in cytoplasmic Ca^{2+} levels have been shown to be responsible for events such as fertilisation and muscle contraction.[2] There are two sources of Ca^{2+} available to increase the cytoplasmic Ca^{2+} concentration; an unlimited extracellular supply and a more finite intracellular store in the endoplasmic and sarcoplasmic reticulum.

1.1.1 Mobilisation of calcium from intracellular stores

The intracellular stores of Ca^{2+} are mobilised from one of two different receptors:

1.1.1.1 Ryanodine receptor

Ca^{2+} Release through sarcoplasmic reticulum Ca^{2+}-release channels (ryanodine receptors) was first observed in 1976[5] but it was not until 1985, when it was found that a plant alkaloid ryanodine bound with high specificity and affinity to these proteins in the sarcoplasmic reticulum, that the isolation and incorporation into planar lipid bilayers of the ryanodine receptor was achieved. The resulting Ca^{2+}-channels were found to be activated by Ca^{2+}, caffeine, adenine nucleotides and inhibited by Mg^{2+} and ruthenium red.[6;7] Further work saw the identification of three different genes in mammals —*ryr1*, *ryr2*, and *ryr3*—encoding skeletal (Ryr1), cardiac (Ryr2), and brain (Ryr3) ryanodine receptor isoforms respectively.[8] Their individual structure and properties have been reviewed in detail[9]and therefore will not be elaborated here.

Of particular note here is the identification of a physiological ligand for the ryanodine receptors located in sea urchin eggs that releases Ca^{2+}. This ligand was identified as a cyclic derivative of nicotinamide adenine dinucleotide (NAD^+) called cyclic ADP-ribose (cADPR, **1**)[10] (Figure **1.1**). The properties of cADPR and its stimulation of Ca^{2+} signalling in several higher eukaryotic cell systems have also been investigated with the suggestion that it acts as a second messenger signalling molecule.[11]

However some aspects of the properties of Ca^{2+} release by cADPR in these systems appear to be different to those originally characterised in sea urchin eggs, suggesting that other target proteins may also be involved in mediating the effects of cADPR.

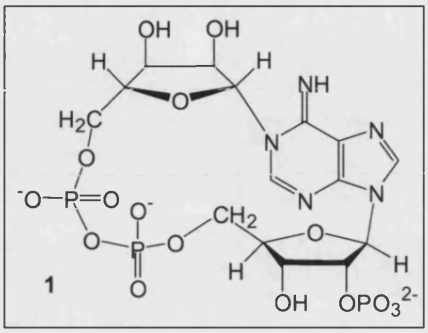


Figure **1.1**: Cyclic ADP-ribose

1.1.1.2 Ins(1,4,5)P_3 receptor

The other intracellular receptor responsible for Ca^{2+} release into the cell cytoplasm is stimulated by D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3][12] and is consequently termed the Ins(1,4,5)P_3 receptor. Formation of Ins(1,4,5)P_3 is mostly regulated by mechanisms in the cell plasma membrane which may be divided into two different categories, those regulated by the GTP binding protein (G-protein) linked receptors, and those regulated by tyrosine kinase receptors. These pathways however both involve the activation of phospholipase C (PLC) and its cleavage of phosphatidylinositol 4,5-bisphosphate [PtIns(4,5)P_2], to Ins(1,4,5)P_3 (**2**) and diacylglycerol (DAG, **3**) (Figure **1.2**).

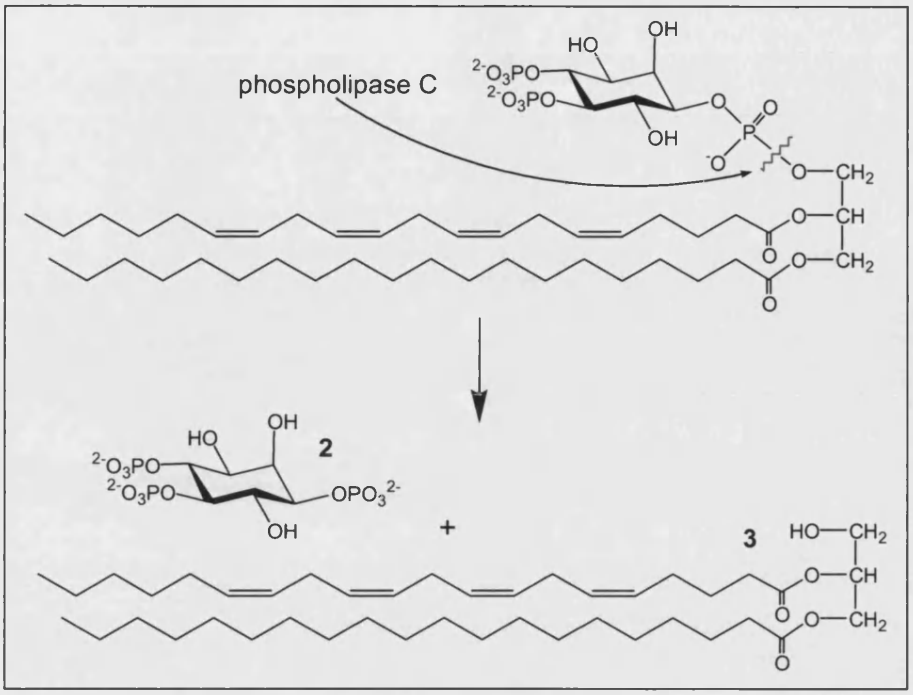


Figure **1.2**: Cleavage of PtIns(4,5)P_2 by phospholipase C to give Ins(1,4,5)P_3 and DAG

1.2.2 G-protein linked plasma membrane receptors

Most G-protein linked receptors have seven transmembrane domains, consisting of hydrophobic amino acid residues. The binding site is located in a pocket near the centre of the lipid bilayer. On binding of a specific ligand to the receptor's extracellular binding site, it undergoes a conformational change, that is transmitted to the cytosolic loops II and III. An associated heterotrimeric G-protein is activated by this conformational change, and dissociates into the G_α and $G_{\beta\gamma}$ subunits. After exchanging GTP for bound GDP, the G_α subunit interacts with PLC-β1, (PLC has three isozymes, PLC-β, PLC-δ and PLC-γ, each of these has more than one subtype of which PLC-β1 is an example). Once activated PLC-β1 interacts with PtIns(4,5)P_2 and causes its cleavage to Ins(1,4,5)P_3 and DAG (Figure **1.3**). Activation of PLC ceases when the GTP associated with the G-protein is hydrolysed to GDP by the intrinsic GTP-ase activity of the G_α subunit which then recombines with the $G_{\beta\gamma}$ subunit, to form the inactive heterotrimeric complex.[12]

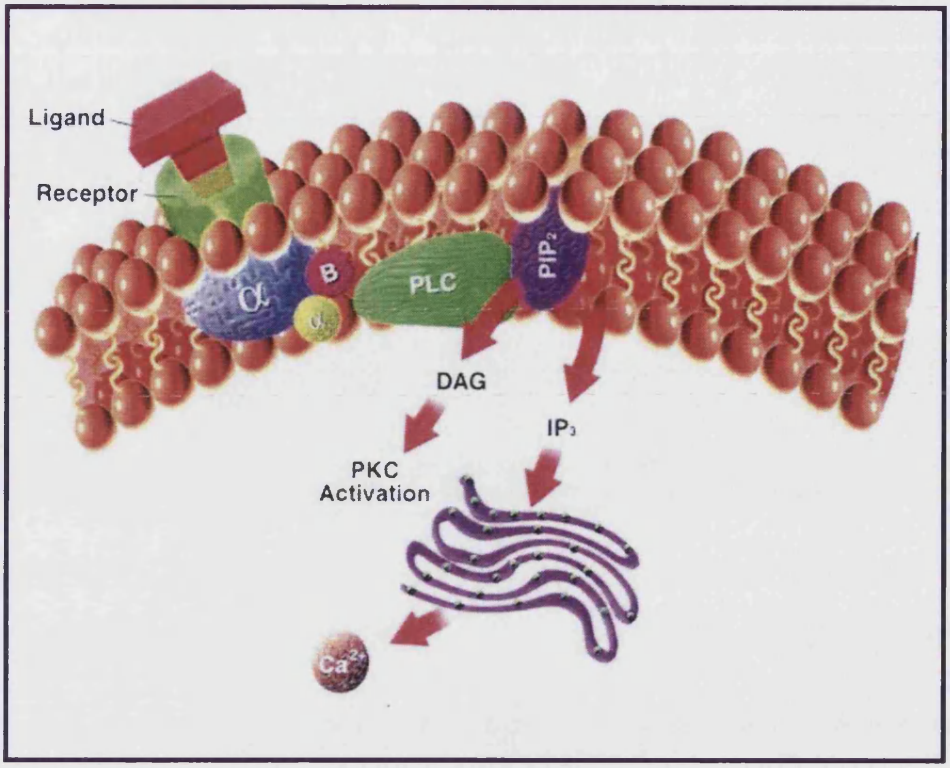


Figure **1.3**: The G-protein linked receptor mediated generation of Ins(1,4,5)P_3 and DAG

1.2.3 Tyrosine kinase plasma membrane receptors

A simpler single transmembrane-spanning domain makes up the tyrosine kinase receptor. Binding of ligands such as platelet-derived (PDGF), and epidermal growth factor (EGF), cause the receptor to dimerise, allowing the cytoplasmic domains of the two receptors to phosphorylate each other on tyrosine residues. This forms a docking site for the SH2 domain of PLC-γ1, which on translocation from the cytosol, binds and is phosphorylated on specific tyrosine residues.[12] Localisation from the cytosol to the membrane brings PLC-γ1 into close proximity with PtIns(4,5)P_2, providing the conditions for its cleavage into Ins(1,4,5)P_3 and DAG.[4]

It has also been shown that signalling from tyrosine kinase receptors through PLC-γ1 may involve stimulation of PtIns(4,5)P_2 3-kinase and generation of PtIns(3,4,5)P_3, which could contribute, directly or indirectly, to a growth factor stimulation of PLC activity and subsequent Ins(1,4,5)P_3 formation.[13] Other cell responses to receptor stimulation that appear to be regulated by PtIns(3,4,5)P_3 formation include: i) insulin-stimulated glucose uptake by target tissues, notably adipose and skeletal muscle;[14] ii) the maintenance of cell survival in response to survival factors such

as insulin-like growth factor;[15] and iii) activation of lamellipodia formation on the leading edge of migrating cells.[14]

The two cleavage products of PtIns(4,5)P_2, both have roles as second messengers. DAG itself has two possible functions; it may be cleaved to release arachidonic acid, which either acts as a messenger in its own right or may be used in the synthesis of eicosanoids. But more importantly for cell signalling, DAG can activate a family of protein kinase C isozymes, which catalyse protein phosphorylation.[16]

1.2 The Ins(1,4,5)P_3 Receptor, its structure and regulation

Ins(1,4,5)P_3 diffuses into the cell cytosol where it binds to Ins(1,4,5)P_3 receptors. By far the greatest concentration of receptors are found in the membrane of the endoplamic reticulum (ER). Ins(1,4,5)P_3 Receptors have also been found in the plasma membrane of Jurkat T-lymphocytes,[17;18] epithelial cells,[19] and Purkinje cells;[20] and not surprisingly, since the ER is contiguous with the outer membrane of the nuclear envelope, Ins(1,4,5)P_3 receptors have been found there too.[21]

The presence of a family of Ins(1,4,5)P_3 receptors has now been established. Three mammalian receptor isoforms are known,[22] corresponding to three distinct Ins(1,4,5)P_3 receptor encoding genes. The tissue distribution studies carried out so far show that all cells contain multiple receptor isoforms but that one isoform may be predominant in a particular tissue or cell type (Table **1.1**). Isolated Ins(1,4,5)P_3 receptor protein proved to be a tetramer, forming an Ins(1,4,5)P_3-gated ion-channel.[22] At least four genes code for the different Ins(1,4,5)P_3 receptor isoforms, all of which share significant similarity to each other, have partial homology with the ryanodine receptor (discussed previously), and no significant homology with voltage-dependent Ca^{2+} channels.[4]

Finally it has been found that different Ins(1,4,5)P_3 receptor isoforms can combine to form heteroligomers, complicating the study of Ins(1,4,5)P_3 signalling even further. This was shown experimentally by a single isoform-specific antibody co-immnoprecipitating additional isoforms from cell extracts.[22]

Family	Predominant tissue distribution
Type-1	Brain, A7r5 smooth muscle cells, uterus, peripheral tissue
Type-2	lung, hepatocytes, testis, spleen
Type-3	intestine, kidney, pancreatic islets

Table **1.1**: Location of $Ins(1,4,5)P_3$ receptor isoforms.

Several subtype-specific properties of $Ins(1,4,5)P_3$ receptors have been identified and these have been discussed in great detail in a review by Patel *et al.*[23]

Each of the four subunits of the $Ins(1,4,5)P_3$ receptor consists of three domains. Contained in the large amino terminal portion of the receptor, which projects into the cytoplasm, there is the $Ins(1,4,5)P_3$ binding domain, and the regulatory domain. Located near the carboxy terminus and embedded in the ER membrane is the transmembrane domain, which forms the Ca^{2+} channel (Figure **1.4**).

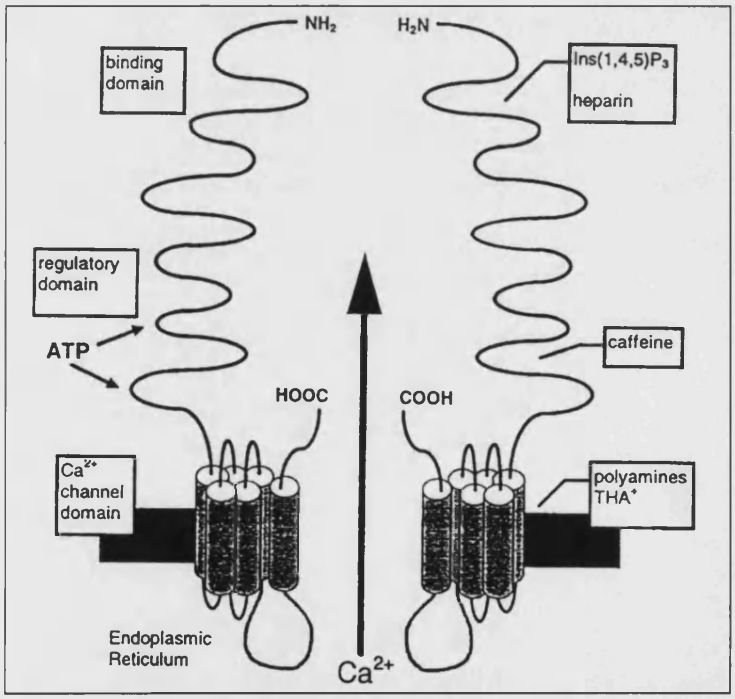


Figure **1.4**: Cross section of a generalised $Ins(1,4,5)P_3$ receptor, showing proposed site of action of some antagonists.[24] THA^+=tetrahexylammonium ion

1.2.1 The $Ins(1,4,5)P_3$ receptor binding domain

The binding affinity of $Ins(1,4,5)P_3$ for the N-terminal segment varies between different receptor isoforms. Two different groups have found different rank orders for

the binding affinity of Ins(1,4,5)P_3 to the receptor isoforms. Using monomeric N-terminus fusion proteins of the Ins(1,4,5)P_3 receptor Newton *et al.*[25] found affinities in the following order: type-2 > type-1 > type-3. Meanwhile Wojcikiewicz and Luo[26] utilised subtype-specific antibodies to immunoprecipitate pure homotetrameric Ins(1,4,5)P_3 receptor populations, and they found a rank order of type-1 ≈ type-2 > type-3.

The antagonists heparin and decavanadate are believed to act at the Ins(1,4,5)P_3 binding site, although the usefulness of heparin as a competitive antagonist has its limitations,[27;28] as it has also been reported to stimulate L-type Ca^{2+} channels,[29] uncouple receptors from their G-proteins,[30] inhibit the kinase responsible for Ins(1,3,4,5)P_4 formation,[31] inhibit binding of Ins(1,3,4,5)P_4,[32] and stimulate Ca^{2+} release through the ryanodine receptor.[33]

1.2.2 The Ins(1,4,5)P_3 receptor regulatory domain

Physiological control over the Ins(1,4,5)P_3 receptor occurs at the regulatory domain where Ca^{2+} interacts and phosphorylation occurs. It has been noted that the homology between different Ins(1,4,5)P_3 receptor subtypes is the lowest in the regulatory domain, suggesting that differential regulation may occur amongst different subtypes.[34] The regulation of ligand binding by Ca^{2+} is complex and tissue dependent.[33] Phosphorylation of the regulatory domain by A-kinase,[35] G-kinase,[36] and tyrosine kinase[37] has been observed in intact cells. Studies carried out in the liver have found that the effect of A-kinase phosphorylation is to potentiate the action of Ins(1,4,5)P_3 in hepatocytes.[35] However other investigations propose that the activation of G-kinase, resulting from increased cGMP levels, is to inhibit Ca^{2+} release. Since the level of nitric oxide appears to mediate increased cGMP levels it is concluded that ultimately nitric oxide has an inhibitory effect on Ca^{2+} release.[38] The regulatory domain also has two binding sites for ATP, and caffeine is thought to be a non-competitive antagonist acting at these binding sites.[27] In addition it has recently been demonstrated that neither adenophostin A nor cADPR bind to these sites.[39]

The ubiquitous Ca^{2+}-binding protein calmodulin has been shown to modulate the mobilisation of Ca^{2+} from cerebellar microsomes. Each Ins(1,4,5)P_3 receptor subunit was found to bind two molecules of calmodulin in the presence of Ca^{2+} and one in its absence[40]. Binding of calmodulin was shown to decrease the affinity of the receptor for

$Ins(1,4,5)P_3$, and hence markedly reduce the potency of $Ins(1,4,5)P_3$ (by *ca.* 10-fold) in mobilising Ca^{2+} from cerebellar microsomes. This effect was independent of the Ca^{2+} concentration.[41]

Another protein which has been found to be tightly associated with the $Ins(1,4,5)P_3$ receptor is FKBP12 (FK506-binding protein). Treatment with FK506 disrupts this association and dramatically increases the Ca^{2+} releasing ability of $Ins(1,4,5)P_3$ from cerebellar microsomal preparations.[42]

1.2.3 The $Ins(1,4,5)P_3$ receptor transmembrane domain

The calcium channel domain is currently thought to form six transmembrane helices, with a proposed hydrophobic hairpin loop between the fifth and sixth helices. This loop is probably embedded in the membrane where it could be part of the ion-conducting pore. The ion-channel seems to exhibit poor discrimination between divalent and monovalent cations, and experiments have demonstrated that this channel behaves as a single file ion pore.[22] Polyamines are able to inhibit Ca^{2+} release from the $Ins(1,4,5)P_3$ receptor with the potency of a polyamine being directly related to the number of positive charges on the molecule. It is thought that as a result of their charged nature they may block Ca^{2+} release by interacting with the negatively charged amino acids found in the Ca^{2+} channel.[27]

1.2.4 Properties of Ca^{2+} release

When treated with suboptimal doses of $Ins(1,4,5)P_3$ it was found that only fractions or "quanta" of calcium were released from the cellular calcium stores; only when higher levels of ligand are used does the full calcium store become accessible.[43] Proposed mechanisms for this type of release either involve heterogeneity of the receptors expressed in the cell, differing receptor sensitivity to $Ins(1,4,5)P_3$, or both.[27]

Progress in imaging technology has also allowed the visualisation of Ca^{2+} release in individual cells. It is now clear that Ca^{2+} signals often have different spatiotemporal properties, thereby eliciting different physiological outcomes.[2;44] Localised signals referred to as elementary events, such as Ca^{2+} puffs or sparks, are dependent on the nature and location of the $Ins(1,4,5)P_3$ receptors involved. It has been found that these elementary events can either activate highly localised cellular processes in the vicinity of

the channels, or by recruiting channels throughout the cell, they can activate either a global Ca^{2+} wave inside the cell or a global Ca^{2+} wave across several cells.[45]

Ca^{2+} Release event	Examples of physiological effects
Elementary event	mitochondrial metabolism membrane excitability vesicle secretion smooth muscle relaxation
Global Ca^{2+} wave (intracellular)	fertilisation smooth muscle contraction liver metabolism cell proliferation
Global Ca^{2+} wave (extracellular)	wound healing ciliary beating insulin secretion bile flow

Table **1.2**: Spatial aspects of Ca^{2+} signalling adapted from Berridge, Bootman, *et al.*[2]

1.2.5 The modulation of Ins(1,4,5)P_3 receptors by Ca^{2+}

The most immediate effects of released Ca^{2+} on the surrounding Ins(1,4,5)P_3 receptors and the current knowledge of the mechanisms involved have been reviewed recently.[46] It has been established that the regulation of the Ins(1,4,5)P_3 receptors by Ca^{2+} in most cells is biphasic, with low concentrations of Ca^{2+} resulting in rapid stimulation of the receptors, and higher concentrations resulting in a slower inhibition.[47] Taylor proposes that the mechanism for the regulation involves binding of Ins(1,4,5)P_3 to an Ins(1,4,5)P_3 receptor subunit binding domain, which causes a large conformational change that both exposes a Ca^{2+}-binding site and initiates the inactivation process. This leads to the receptor binding Ins(1,4,5)P_3 with increased affinity and becoming less capable of opening to its fully active state. It is thought that it is only when all four subunits of the receptor have bound Ins(1,4,5)P_3 and Ca^{2+} in their respective binding sites that the channel pore opens. It is then thought that as the cytosolic [Ca^{2+}] increases it binds to a second binding site on the Ins(1,4,5)P_3 receptor resulting in the relatively slow inhibition of channel opening.

1.3 PH domains

1.3.1 Structure

The term Pleckstrin Homology (PH) domain was first used in 1993 to describe homologous regions of approximately 120 amino acids found in a number of proteins involved in functions such as intracellular signalling and cytoskeletal organisation. These regions or domains were termed PH domains because the domain was first identified in the phosphoprotein pleckstrin.

Although the primary sequence of PH domains varies considerably, their secondary structure is quite similar, this being one of the criteria for a PH domain. They comprise seven β-strands and a C-terminal α-helix. The loops between these strands are variable in length and sequence. Furthermore NMR spectroscopy and X-ray crystallography has revealed a common tertiary structure. Each domain is a β-sandwich formed by two nearly orthogonal antiparallel β-sheets comprising four and three β-strands respectively, and the C-terminal α-helix closes one end of the β-sheets. It has been proposed that the diversity in the primary structure of these domains provides the means for their functional diversity that is currently receiving a great deal of attention.

1.3.2 Function

In 1994 Harlan *et al.*[48] reported that the PH domains of several proteins including pleckstrin, β-adrenergic receptor kinase (βARK), *ras*-GTP-activating protein and T-cell specific-kinase, bound phosphatidylcholine vesicles containing PtdIns(4,5)P_2 *in vitro*. Moreover it was shown that a large excess of Ins(1,4,5)P_3 could inhibit the phosphoinositide-PH domain interaction. Since then, these and other proteins have been found to bind a number of different phosphoinositides and inositol phosphates. These findings are summarised in Table **1.3**.

PH domain	Proposed ligand
pleckstrin	PtdIns(4,5)P_2
β-spectrin	g-PtdIns(4,5)P_2*
PLC-δ_1	Ins(1,4,5)P_3 PtdIns(4,5)P_2
PLC-γ	Ins(1,3,4,5)P_4
p130	Ins(1,4,5)P_3 Ins(1,4,5,6)P_4
RAC/PKB/Akt	PtdIns(3,4,5)P_3 PtdIns(3,4)P_2 Ins(1,4,5,6)P_4/Ins(1,3,4,5,6)P_5
Btk	Ins(1,3,4,5)P_4 PtdIns(3,4,5)P_3
Dynamin	PtdIns(4,5)P_2
Sos	PtdIns(4,5)P_2 PtdIns(3,4,5)P_3
Diacylglycerol kinase	Ins(1,3,4,5,6)P_5
β-ARK	Ins(1,4,5)P_3

Table **1.3**: Summary of proposed ligands for PH domains.[49;50]

Of particular interest is the interaction of PLC-δ_1 with Ins(1,4,5)P_3 and PtdIns(4,5)P_2. Antibody-tagged fluorescence studies have been used to show that PLC-δ_1 requires its PH domain for membrane attachment. Single amino acid substitutions in the PH domain of PLC-δ_1 have been shown to affect its ligand binding, phospholipase and membrane binding activities. Furthermore after agonist stimulation green fluorescent protein-labelled PH domains of PLC-δ_1 have been shown to localise at the plasma membrane and then transiently become cytosolic upon depletion of PtdIns(4,5)P_2. Consideration of these results has led to the suggestion that the PLC-δ_1 PH domain serves to anchor PLC-δ_1 to the plasma membrane *via* a PtdIns(4,5)P_2-PH domain interaction. This is followed by the cleavage of PtdIns(4,5)P_2 to Ins(1,4,5)P_3 and DAG. Ins(1,4,5)P_3 is then thought to compete with PtdIns(4,5)P_2 for the PH domain binding site, resulting in the release of the enzyme from the plasma membrane. It is thought that this regulation of the PH domain interaction with PtdIns(4,5)P_2 may

* g-PtdIns(4,5)P_2 is 1-(α-glycerophosphoryl)inositol 4,5-bisphosphate

represent an important feedback mechanism by which excessive PtdIns(4,5)P_2 hydrolysis by PLC-δ_1 is avoided.

Previous discussion has implicated PtdIns(4,5)P_2 3-kinase activity in the regulation of PLC-γ_1. The role of PtdIns(4,5)P_2 3-kinase has been investigated with the use of confocal fluorescence microscopy and green fluorescent protein-labelled PLC-γ_1 PH domain[51]. It was demonstrated that PtdIns(4,5)P_2 3-kinase activity can induce membrane localisation of the PLC-γ_1 PH domain *in vivo*. Such localisation is thought to result in the cleavage of PtdIns(4,5)P_2 to give Ins(1,4,5)P_3 and DAG. Such results suggest that PtdIns(4,5)P_2 3-kinase plays a role in the formation of Ins(1,4,5)P_3 catalysed by PLC-γ1.

1.3.3 X-ray crystal structures of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 bound to PH domains

The X-ray crystal structure of Ins(1,4,5)P_3 bound to the PH domains of β-spectrin[52] and PLC-δ_1[53] were reported in 1995. This has been followed by the publication of the X-ray crystal structure of Ins(1,3,4,5)P_4 bound to the PH domain of Bruton's tyrosine kinase.[54]

The two PH domain-Ins(1,4,5)P_3 complexes were found to be quite different. To begin with Ins(1,4,5)P_3 bound to different regions of the respective PH domains. In β-spectrin Ins(1,4,5)P_3 was bound between loops β-1/2 and β-5/6, while in PLC-δ_1 Ins(1,4,5)P_3 bound to residues from loops β-1/2 and β-3/4. Figure **1.5** shows a schematic representation of the key residues involved in holding Ins(1,4,5)P_3 at the respective binding sites. It is immediately clear that there are large differences in the number and arrangement of interactions, which probably accounts for the differences in stability of the two complexes. The complex formed between Ins(1,4,5)P_3 and the PH domain of PLC-δ_1 was 200-fold more stable than that formed between Ins(1,4,5)P_3 and β-spectrin. The most noticeable difference between the two complexes concerns the Ins(1,4,5)P_3 5-phosphate, which has twice the number of hydrogen bonds to amino acid residues in the PLC-δ_1 complex, than in the β-spectrin complex.

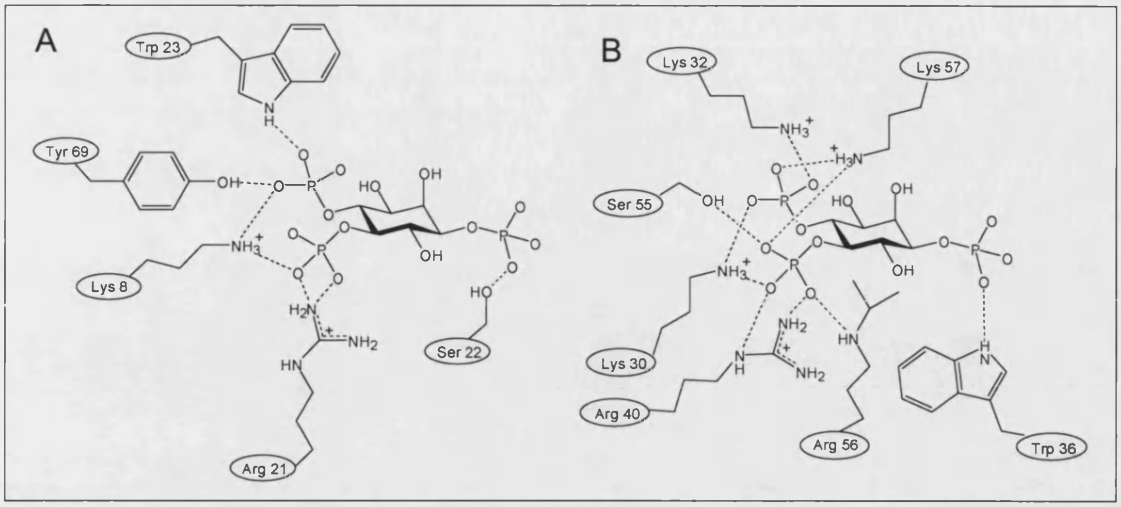


Figure **1.5**: Schematic representation of the Ins(1,4,5)P_3 binding site of A) β-spectrin and B) PLC-δ_1 PH domains. Adapted from Hyvönen *et al.*[52] and Ferguson *et al.*[53] respectively.

A schematic representation of the binding site of Ins(1,3,4,5)P_4 in the PH domain of Bruton's tyrosine kinase is depicted in Figure **1.6**, like the PLC-δ_1 Ins(1,4,5)P_3 complex, the Ins(1,3,4,5)P_4 binding site was found between the β-1/2 and β-3/4 loops of the PH domain. The PH domain of Bruton's tyrosine kinase has been shown to exhibit specificity for Ins(1,3,4,5)P_4 over Ins(1,4,5)P_3 and the reason for this preference was clear from the crystal structure, in which the 3-phosphate was found to be held by a significant number of hydrogen bonds to the amino acid residues of the PH domain. The report by Baraldi *et al.*[54] also investigated the binding of Ins(1,3,4,5)P_4 to mutants of the Bruton's tyrosine kinase PH domain, since they have been associated with an X chromosome linked immunodeficiency caused by a mutation in the murine Btk gene. They concluded that the mutant phenotype has different causes, with different mutations resulting in different structural changes in the PH domain and thus affecting Ins(1,3,4,5)P_4 binding to the PH domain in different ways.

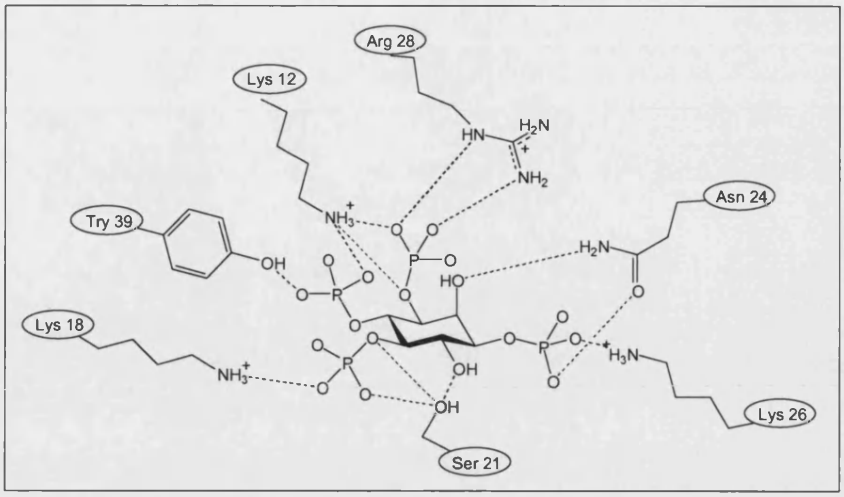


Figure **1.6**: Schematic representation of the Ins(1,3,4,5)P_4 binding site of the Bruton's tyrosine kinase PH domain. Adapted from Baraldi *et al.*[54]

The preceding examples of PH domain function in known phosphoinositide signalling pathways, together with the X-ray crystal structures of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 bound in PH domains are just part of the research in this field. As a result of the great interest in PH domains and their interactions with other cellular molecules the rate of progress in understanding their role in cellular signalling has been, and continues to be, very rapid. The current knowledge pertaining to the phosphoinositide signalling pathway has been discussed here, but for a more detailed consideration of PH domain-phosphoinositide interactions the reader is referred to two comprehensive reviews published within the last year.[49;50]

1.4 Mechanisms for entry of extracellular Ca^{2+} into the cell

Entry of extracellular Ca^{2+} through the plasma membrane into the cell occurs through three different channels: i) voltage-operated Ca^{2+} channels (VOCs) in excitable cells such as neurones or muscle cells, ii) receptor-operated Ca^{2+} channels (ROCs) in response to neurotransmitters, and iii) store-operated Ca^{2+} channels (SOCs) which open when the intracellular Ca^{2+} stores have been depleted and are mainly found in non-excitable cells. These channels mainly function to provide Ca^{2+} for increases in the cytoplasmic free Ca^{2+} concentration and to replenish Ca^{2+} stores in the sarcoplasmic and ER which have been released and subsequently pumped out of the cell.

1.4.1 Capacitative Ca^{2+} entry

It has been found that depletion of the intracellular stores of Ca^{2+} following Ca^{2+} release through the Ins(1,4,5)P_3 receptor, activates a mechanism by which the SOCs in the plasma membrane are opened to allow extracellular Ca^{2+} into the cell[55] (Figure **1.7**). This phenomenon, termed the *capacitative model* for Ca^{2+} entry, has been demonstrated through the depletion of stores with both phospholipase C-linked agonists and specific experiments with inhibitors of intracellular Ca^{2+} transport ATPases that mediate the active accumulation of Ca^{2+} in the endoplasmic and sarcoplasmic reticulum. A particularly effective example of such an inhibitor is thapsigargin;[56] when applied to cells it results in a relatively rapid passive depletion of intracellular Ca^{2+} stores. Although this depletion has been shown to induce the opening of SOCs, the mechanism by which it does so remains unclear. Consequently several hypotheses have been proposed for the mechanism of activation of SOCs and these have been summarised in the table below since the subject has been extensively reviewed by several authors.[57-61] Also of note here is a recent review by Shuttleworth challenging the capacitative model of Ca^{2+} entry.[62]

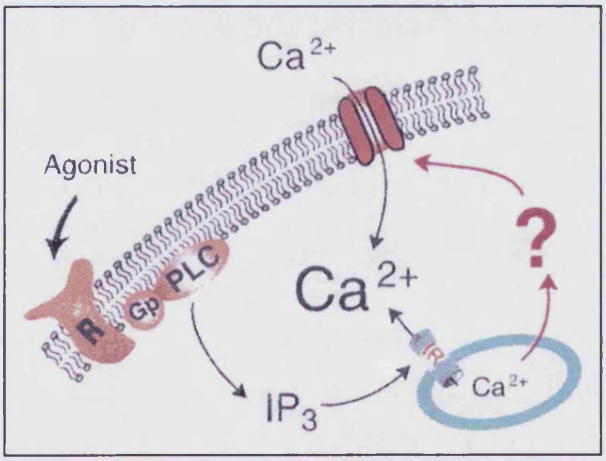


Figure **1.7**: Capacitative Ca^{2+} entry. Agonist activation of a surface receptor (R) coupled through a G-protein (Gp) to phospholipase C (PLC) leads to the production of Ins(1,4,5)P_3 which causes Ca^{2+} release. The depletion of endoplasmic reticulum Ca^{2+} stimulates the entry of Ca^{2+} through the plasma membrane by an unknown mechanism (red pathway).[58]

Hypothesis	Summary	Comments and references
Conformational coupling	A protein-protein interaction between the Ins(1,4,5)P_3 receptor or another protein in the ER and the SOC protein in the plasma membrane[43;59;63]	Recent experiments suggest that thapsigargin activation of SOC's is independent of Ins(1,4,5)P_3 receptor proteins.[64;65] However interactions between SOC proteins and another ER proteins are possible
Diffusable messenger	A diffusable messenger released by the ER interacts with the SOC to induce Ca^{2+} entry.[66;67]	Suggested candidates include Ca^{2+}-influx factor,[67-69] cytochrome P-450 metabolites[70;71] and arachidonic acid.[72]
Vesicle secretion	The insertion of vesicles rich in SOC proteins into the plasma membrane.[73]	Most unlikely mechanism, probably not universal.[74]
G-Protein coupling	Coupling of the decrease in cytoplasmic Ca^{2+} to the SOC by a monomeric or trimeric G-protein.[75-79]	It is more likely that G-proteins play a more permissive role even though they are necessary for SOC activation in some cell types[80]

Table **1.4**: Summary of the proposed mechanisms of SOC activation adapted from a paper by G. J. Barritt.[81]

Investigations into the flow of Ca^{2+} through the plasma membrane has seen the characterisation of a depletion-activated calcium-selective current (I_{CRAC}), and its relationship to the depletion of intracellular Ca^{2+} stores.[82] It has also been found that a slow feedback inhibition of I_{CRAC} by Ca^{2+} entry exists[83] which accounts for up to 70% of the total inactivation of I_{CRAC}. Furthermore it has been proposed that sphingosine directly blocks I_{CRAC} and it is therefore suggested that the sphingomyelin pathway is involved in I_{CRAC} regulation.[84]

The actual identity of the SOC-protein has also received considerable attention. Some studies have found evidence for the involvement of the type 3 $Ins(1,4,5)P_3$-receptor. One example of such a study demonstrated that the expression of the type 3 $Ins(1,4,5)P_3$-receptor in *Xenopus* oocytes specifically augments Ca^{2+} entry,[85] without affecting Ca^{2+} release. This finding certainly gives credence to the idea of type 3 $Ins(1,4,5)P_3$-receptors acting as SOCs in some instances, but the greatest amount of interest in this area has been in a protein product of the transient receptor potential (*trp*) gene in *Drosophila* photoreceptors. The *Drosophila* photoreceptor mutant termed *trp* is characterised by an inability of the photoreceptors to sustain an influx of Ca^{2+} during intense illumination. These photoreceptors use a phospholipase C signalling system and so it was thought that *trp* may encode a component of the Ca^{2+} entry pathway. Several *trp* proteins have now been identified and although some have clearly demonstrated the mediation and augmentation of capacitative Ca^{2+} entry, their electrophysiology did not match that of known depletion-activated calcium-selective currents (I_{CRAC}) (reviewed [58]). Thus investigation into the properties of this family of proteins and their involvement in capacitative Ca^{2+} entry needs further work to define their true role.

1.5 Metabolism of Inositol phosphates

Once extracellular stimulation ceases, there needs to be a mechanism to turn off the intracellular response and return the cell back to its basal level, ready for the next stimulus. $Ins(1,4,5)P_3$ itself needs to be removed from the cell in some way, and this function is fulfilled by inositol phosphate metabolising enzymes. The main metabolic pathways are outlined in Figure **1.8**, although this is a somewhat simplistic view of what is a complex system of metabolites and metabolising enzymes.

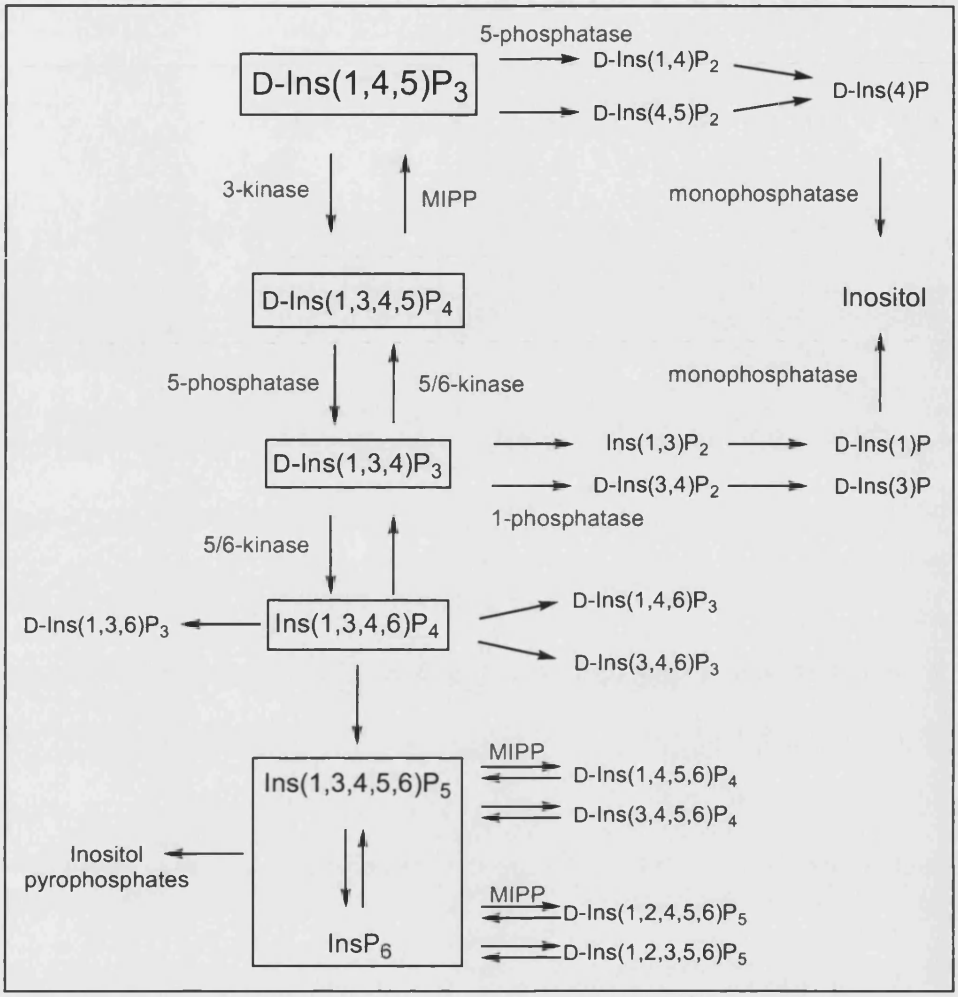


Figure **1.8**: Metabolism of receptor generated $Ins(1,4,5)P_3$ in mammalian cells (MIPP = Multiple Inositol Polyphosphate Phosphatase)

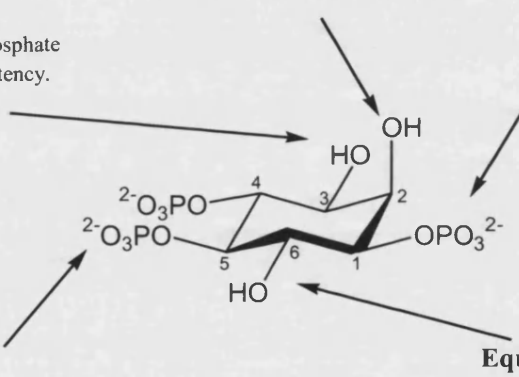
1.6 Structure-Activity Relationships at Ins(1,4,5)P_3 Receptors

Equatorial 3-hydroxyl
Enhances binding, but less important than 6-hydroxyi. May have H-bond interactions with binding site probably via oxygen lone pair electrons.

Deletion or changing to axial for the 6-hydroxyl.
> Replacement with fluorine reduces activity only slightly.
> Axial hydroxyl together withphosphorothioates at 1,4,5 gives a partial agonist.
> Increasing molecular volume with hydrophobic substituents is thought to correlate inversley with potency.
> Equatorial or axial phosphate gives greatly reduced potency.

Axial 2-hydroxyl
The least important part of Ins(1,4,5)P3. Of only minimal importance for binding.
> Can be deleted, changed to equatorial or replaced with fluorine with only slight effect on activity.
> C-2 can be replaced with pyranoside oxygen.
> Bulky substituents or phosphate group are tolerated. Presence of an axial 1-phosphate increases affinity.
> Equatorial 2-OH together with phosphorothioates at 1,4,5 and axial 3 or 6-OH gives low intrinsic activity partial agonists.

Equatorial 1 -phosphate
Less important than the 4- and 5-phosphates. Enhances binding, but not essential. May have a long-range interaction with the receptor binding site.
> Deletion greatly reduces activity, but replacement with axial phosphate or a phosphate more distant from the ring is tolerated.
> large groups can be tolerated without major loss of activity.
> Replacement with phosphorothioate reduces activity only slightly.



4,5-bisphosphate
The most important part of Ins(1,4,5)P3 and essential for activity. Can be replaced with a bisphosphorothioate. Linking the 4- and 5 phosphates to give a cyclic pyrophosphate abolishes activity

Equatorial 6-hydroxyl
Very important for binding. May have H-bond interactions with binding site and/or affect conformation/ionisation state of 5-phosphate, probably via hydroxyl hydrogen rather than lone pair electrons.
> Deletion or changing to axial greatly reduces activity, but in combination with phosphoro-thioate at 1,4,5 gives partial agonists.
> Bulky substituents, equatorial phosphate or replacement with fluorine gives greatly reduced activity.

Equatorial 4-phosphate

Informationis lacking on analogues selectively modified at this position.

> Deletion abolishes activity, changing to axial greatly reduces activity.
> Replacement with phosphorothioate has less effect on potency than replacing the 5-phosphate

Equatorial 5-phosphate

Ionisation state correlates with the affinity of Ins(1,4,5)P_3 for its receptor.

> Deletion abolishes activity, effect of axial not known.
> Can be replaced with methylenephosphonate or phosphorothiate, to give metabolically resistant analogues causing prolonged Ca^{2+} release.

Figure **1.9**: Structure-activity relationships for Ins(1,4,5)P_3 receptors.[24]

Over the past fifteen years there has been a great deal of interest in the synthesis of Ins(1,4,5)P_3 analogues. As a result we now have a good appreciation of the structure-activity relationships of Ins(1,4,5)P_3 binding and potency. These are summarised in Figure **1.9**, and are comprehensively reviewed elsewhere.[86;87]

Since these reviews were written there have been several interesting developments in this field. A ring contracted cyclopentane-based Ins(1,4,5)P_3 analogue (**4**) derived from D-xylose, in which the relative stereochemistry and substitution of positions equivalent to positions 4,5,6 and 1 of Ins(1,4,5)P_3 was retained, has been reported by Jenkins and Potter.[88] This is the latest in a series of cyclopentane Ins(1,4,5)P_3 analogues designed to investigate the tolerance of the Ins(1,4,5)P_3 receptor to a smaller ring size by means of an effective deletion of the 2-position.[89;90] Biological data pertaining to the activity of this analogue have yet to be published.

Remarkable binding affinity for the Ins(1,4,5)P_3 receptor has been described by Takanari *et al.*[91] for a 1-position modified Ins(1,4,5)P_3 analogue (**5**). Modification of this 1-phosphate with the dye molecule malachite green resulted in a compound with a K_d of 1.17 nM in the Ins(1,4,5)P_3-binding domain of human type-1 Ins(1,4,5)P_3 receptor. In the same study the corresponding binding of Ins(1,4,5)P_3 (K_d 195nM) was found to be 167-fold weaker. However, there was no Ca^{2+} release data reported in this paper, and therefore the agonist/antagonist properties of (**5**) cannot be commented upon.

Finally the synthesis and properties of three phosphofluoridate analogues (**6,7,8**) of Ins(1,4,5)P_3 have recently been published[92] with a phosphofluoridate at either the 4 or 5-position or both (Figure **1.10**). This report was particularly interesting as it investigated the ionic interaction of the 4,5 bisphosphate of Ins(1,4,5)P_3 with the Ins(1,4,5)P_3 receptor and the active sites of the two metabolising enzymes, Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3 5-phosphatase. Substitution of one phosphate hydroxyl for fluorine was chosen because fluorine provides a steric bulk similar to that of an ionised hydroxyl and is an acceptor for a hydrogen bond in the same way as an ionised hydroxyl, but cannot be involved in ionic bonding interactions. This investigation found that the 4 and 5-phosphates of Ins(1,4,5)P_3 are independently recognised in the binding process, with the 4-phosphate exhibiting stronger ionic interactions with the receptor than the 5-phosphate.

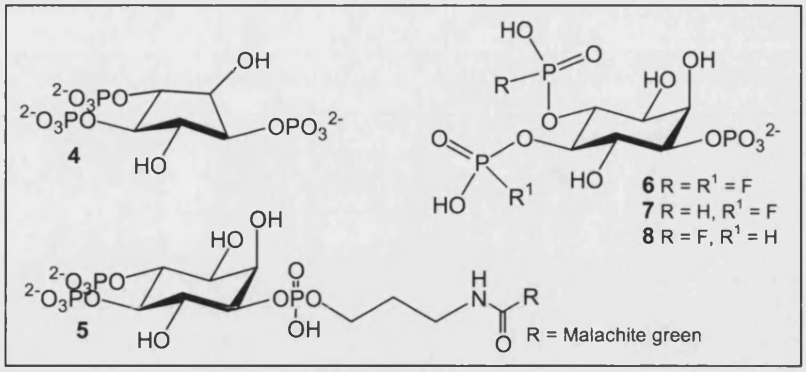


Figure **1.10**: Recently reported Ins(1,4,5)P_3 analogues

1.6.1 Partial agonists

It has been demonstrated that some of the Ins(1,4,5)P_3 analogues that fully displace [^{3}H]Ins(1,4,5)P_3 from its receptor, are not able to mobilise the full Ca^{2+} store. Analogues with such biological properties are referred to as partial agonists. The first analogue found to exhibit this activity was Ins(1,3,4,6)P_4 (**9**). When tested in neuroblastoma cells it mobilised 80-90% of the available Ca^{2+} store.[93]

The introduction of phosphorothioate groups at positions usually substituted with phosphate groups has been used to prepare Ins(1,4,5)P_3 analogues stable to the two Ins(1,4,5)P_3 metabolising enzymes. Two of these phosphorothioate analogues, L-*chiro*-Ins(2,3,5)PS_3 (**10**) and D-6-deoxy-Ins(1,4,5)PS_3 (**11**), have also been reported to exhibit partial agonist activity, fully displacing [^{3}H]Ins(1,4,5)P_3 from its binding sites on adrenal cortical membranes, and yet only mobilising 34% and 42% respectively of Ca^{2+} mobilised by Ins(1,4,5)P_3.[94] Further phosphorothioate-based partial agonists DL-Ins(1,3,4)PS_3 (**12**) and DL-Ins(1,4,6)PS_3 (**13**) were then synthesised and found to release around 30% and 20% of Ca^{2+} mobilised by Ins(1,4,5)P_3[95].

D-3-Amino-3-deoxy-*myo*-Ins(1,4,5)P_3 (**14**) is an unusual partial agonist, in that it exhibits pH dependence, behaving as a full agonist at pH 7.2 and 7.6, and behaving as a partial agonist at pH 6.8, releasing about 80% of Ins(1,4,5)P_3 mobilisable Ca^{2+}.[96]

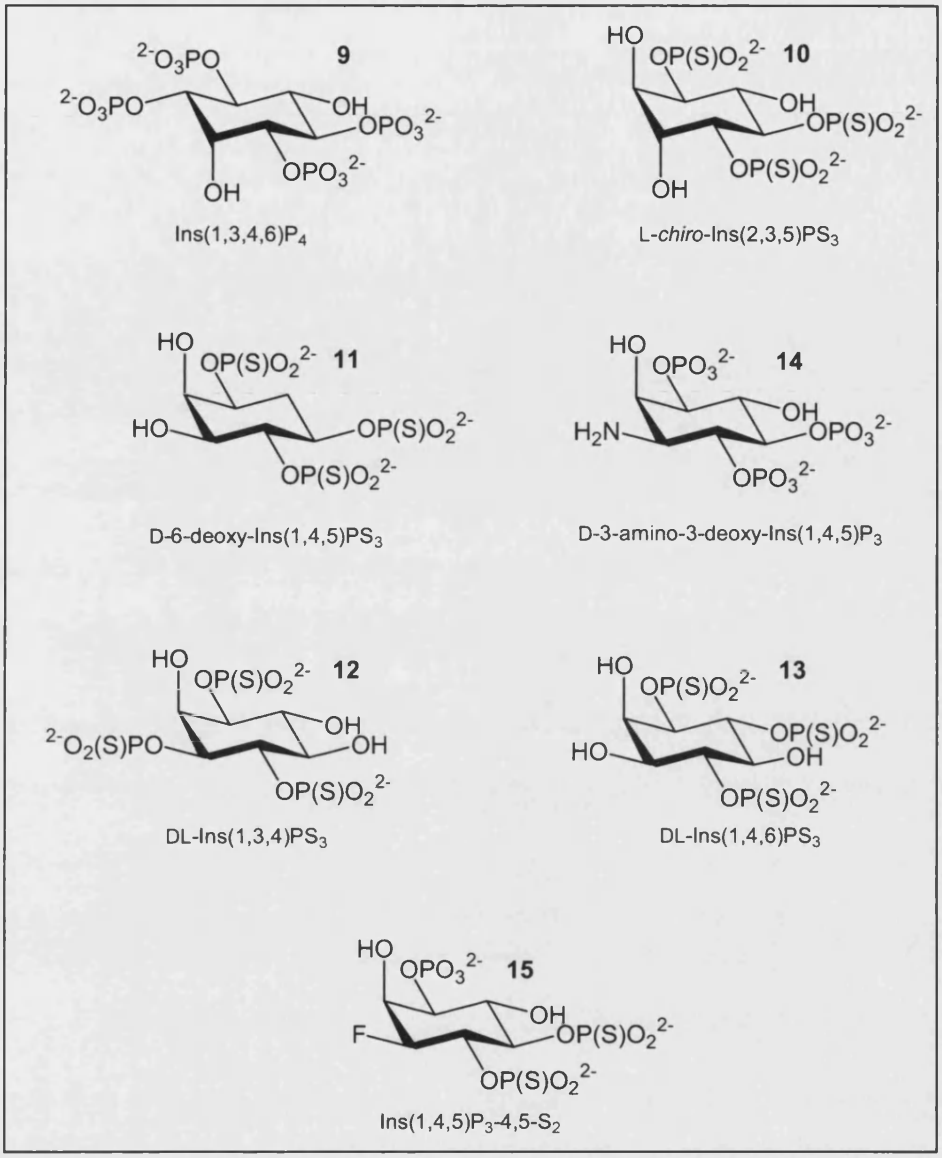


Figure **1.11**: Class I partial agonists at Ins(1,4,5)P_3 receptors.

From the biological activity of the above Ins(1,4,5)P_3 analogues it was proposed that structural perturbation at the 3-position combined with phosphorothioate substitution at the 4 and 5-positions of Ins(1,4,5)P_3 were the key elements to partial agonist activity. This was investigated by Fauq *et al.*[97] who reported the synthesis of D-3-deoxy-3-fluoro-*myo*-inositol 1-phosphate-4,5-bisphosphorothioate (**15**) along with D-3-deoxy-3-fluoro-*myo*-inositol 1,5-bisphosphate-4-phosphorothioate and D-3-deoxy-3-fluoro-*myo*-inositol 1,4-bisphosphate-5-phosphorothioate. The bisphosphorothioate was indeed found to be a partial agonist when evaluated in SH-SY5Y cells, with both the monophosphorothioates acting as full agonists, the most potent of these being the 4-phosphorothioate.[97;98]

At a similar time another report described the partial agonist behaviour for 3-deoxy-*myo*-inositol 1,4,5-trisphosphate (**16**), 2,3-dideoxy-*myo*-inositol 1,4,5-trisphosphate (**17**), 2,3,6-trideoxy-*myo*-inositol 1,4,5-trisphosphate (**18**) and *myo*-inositol 2,4,5-trisphosphate (**19**)[99] (Figure **1.12**). Although these analogues were shown to mobilise the full Ca^{2+} store, they did so at a slower rate than Ins(1,4,5)P_3. This activity was defined as class II partial agonism, with the analogues that cannot mobilise the full Ca^{2+} store being defined as class I partial agoinists, to avoid confusion.

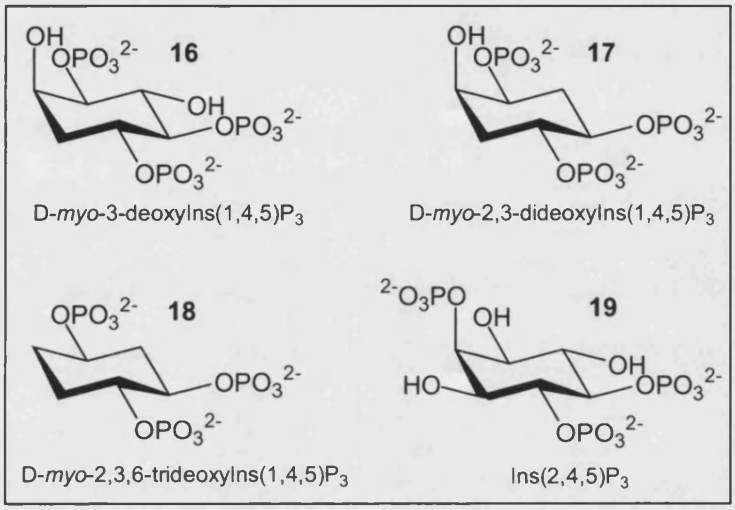


Figure **1.12**: Class II partial agonists at Ins(1,4,5)P_3 receptors.

1.6.2 Antagonists

Heparin and decavanadate have been the only known Ins(1,4,5)P_3 antagonists at the Ins(1,4,5)P_3 receptor for several years. But as mentioned previously they are relatively non-selective and therefore not ideal for use as pharmacological agents.

Recently, however, Gafni *et al.*[100] reported the antagonist activity at the Ins(1,4,5)P_3 receptor of a series of membrane permeable xestospongins, isolated from Australian sponges of the *Xestospongia* species. The most potent xestospongin tested was Xestospongin-C (**20**) which inhibited Ins(1,4,5)P_3-dependent Ca^{2+} release with an IC_{50} of ~0.3μM in rat cerebellar microsomes. These compounds however block Ca^{2+} release from the ryanodine receptor with a potency 30 times lower than that at the Ins(1,4,5)P_3 receptor.

The activity of another antagonist at the Ins(1,4,5)P_3 receptor, 2-aminoethoxydiphenyl borate (2APB, **21**) has also been described.[101] This membrane-permeable compound was reported as a modulator of the Ins(1,4,5)P_3-induced Ca^{2+}

release. 2APB was shown to inhibit [^{3}H]Ins(1,4,5)P_3 binding to its receptor in cerebellar microsomal preparations, although the IC_{50} concentration of 2APB (42μM) for inhibition of Ins(1,4,5)P_3 (100nM) induced Ca^{2+} release, with further increases in 2APB concentration resulting in corresponding increases in Ca^{2+} release.

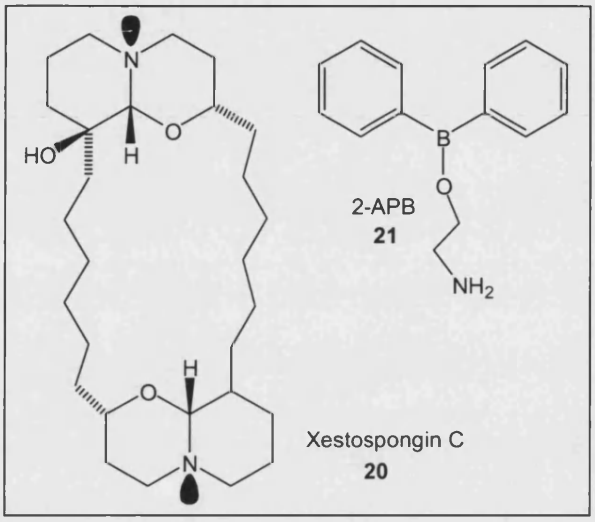


Figure **1.13**: The structure of two antagonists at the Ins(1,4,5)P_3 receptor.

Both the xestospongins and 2APB (Figure **1.13**) are thought to bind to the Ins(1,4,5)P_3 receptor at a site other than the Ins(1,4,5)P_3 binding site, since they did not affect [^{3}H]Ins(1,4,5)P_3 binding to the receptor. They are therefore non-competitive agonists of the Ins(1,4,5)P_3 receptor.

Ca^{2+} Release from the Ins(1,4,5)P_3 receptor has also been blocked by antibodies. For example a paper published within the last few months by Matsuki *et al.*[102] describes the inhibition of binding of Ins(1,4,5)P_3 to its receptor binding site by an antibody against a peptide sequence found in the PH domain of PLC-δ1. Further investigation suggested that it was the Fab fragment of the antibodies that was responsible for inhibiting the binding of Ins(1,4,5)P_3 to its receptor, although treatment of permeabilised rat basophilic leukemic cells with the Fab fragment failed to completely inhibit Ca^{2+} release from the Ins(1,4,5)P_3 sensitive stores of the ER. The main draw back with this approach to Ins(1,4,5)P_3 receptor blockade is that antibodies are unable to cross the plasma membrane.

1.7 The synthesis of inositol phosphates

1.7.1 From inositol

Several problems are inherent in the preparation of inositol polyphosphates from inositol starting materials:

I) Regioselective protection of hydroxyls.

II) Resolution or desymmetrisation of enantiomeric intermediates.

III) Phosphorylation of free hydroxyls.

IV) Deprotection of hydroxyls and protected phosphates.

V) Purification of the free inositol polyphosphate.

The manner in which these issues are currently addressed has been reviewed in detail elsewhere.[86] But they are briefly considered here too, since all but point II) are also relevant to the synthesis of the compounds in this thesis, (the compounds described in this thesis are almost all made from chiral starting materials, therefore no resolution step was necessary).

An elegant example of the preparation of an inositol polyphosphate is the synthesis of *myo*-inositol-1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] (shown in Figure **1.14**), a metabolite of Ins(1,4,5)P_3, by Riley *et al.*[103] Its synthesis demonstrates the techniques used to overcome the difficulties outlined above.

Using *myo*-inositol (a *meso* compound) as the starting material initial regioselective protection of three of the hydroxyl groups was achieved with an orthoformate to give (**22**), which was subsequently desymmetrized by the regioselective introduction of two camphanate esters. Thus in one step the molecule was desymmetrised, since separation of the resulting diastereoisomers gave the D and L isomers **23a** and **23b**. After cleavage of the orthoformate the four exposed hydroxyl groups were phosphorylated using P (III) methodology. This involved phosphitylation with bis(benzyloxy)(diisopropylamino) phosphine activated as the tetrazolide followed by oxidation with MCPBA to the phosphate. A two step deprotection was then carried out. The advantage of this approach to deprotection was that once deprotected the free phosphates were not able to migrate. Finally two methods of purification of the enantiomers of Ins(1,3,4,5)P_4 (**26a** and **26b**) were reported. They were either isolated as the cyclohexylammonium or potassium salts or subjected to ion exchange chromatography to give the triethylammonium salts.

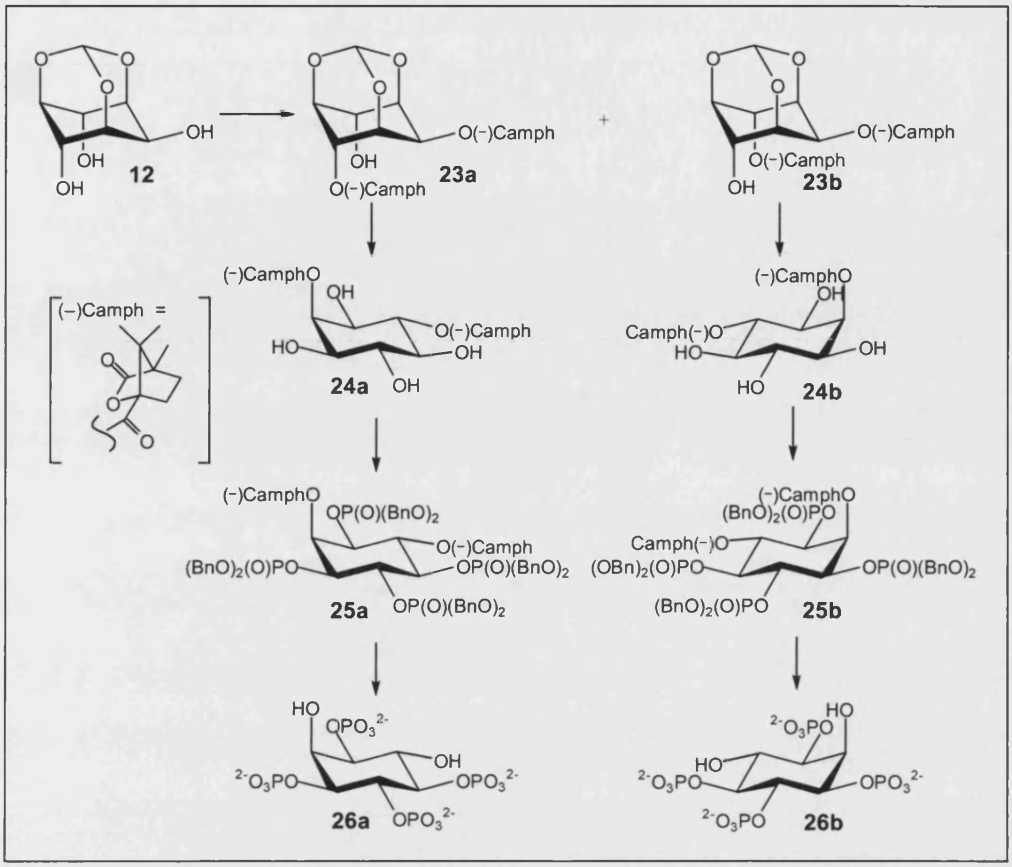


Figure **1.14**: Synthesis of D-Ins(1,3,4,5)P_4 (**26a**) and L-Ins(1,3,4,5)P_4(**26b**).

1.7.2 From monosaccharides

The synthesis of inositol intermediates from simple monosaccharide starting materials is also possible, and several have been reported in the literature. The first synthesis of optically active inositol derivatives from carbohydrate starting materials was reported in 1987 by Watanabe *et al.* from D-glucurono-6,3-lactone.[104] Since then inositol intermediates and polyphosphates have been prepared from D-glucose[105-107] and D-galactose.[108-110] Although the starting materials for such syntheses are cheap and readily available, the vast majority of inositol polyphosphates are still made in the conventional way from inositol.

When carbohydrates are used in the synthesis of inositol intermediates there is no need for a resolution or desymmetrisation step which can often be difficult, tedious and expensive. This is a distinct advantage in the synthesis of Ins(1,4,5)P_3 analogues (although, in some instances the L-enantiomer of an inositol polyphosphate may also be

required, as in the case of *myo*-inositol 1,3,4-trisphophate[111]), therefore the preparation Ins(1,4,5)P_3 analogues based on carbohydrate starting materials is still very attractive.

1.8 Adenophostin A and B

Discovery and Biological activity

Despite all the synthetic interest in Ins(1,4,5)P_3 since 1983, none of the Ins(1,4,5)P_3 analogues prepared so far has had greater potency than Ins(1,4,5)P_3 itself. However, a new development came in 1993 when two new molecules, adenophostin A and adenophostin B, were isolated from the cultured broth of *Penicillium brevicompactum* SANK 11991 and SANK 12177.[112] These two molecules attracted considerable interest because the Ca^{2+} release from cerebellar microsomes by the adenophostins was demonstrated to be 100-fold greater than that of Ins(1,4,5)P_3.[112] (A subsequent paper reported an almost 10-fold increase in Ca^{2+} releasing activity from the type 1 Ins(1,4,5)P_3 receptor in artificial membrane vesicles[113]). In a competition assay, both adenophostins were found to inhibit [^{3}H]Ins(1,4,5)P_3 binding in a more potent manner than Ins(1,4,5)P_3 with a high positive cooperativity.[113] The results also suggested that binding of at least four molecules of adenophostin to each type I Ins(1,4,5)P_3 receptor were needed for Ca^{2+} release. Further observations indicated that suboptimal concentrations of adenophostin, released submaximal amounts of Ca^{2+}; this is indicative of the quantal Ca^{2+} release behaviour of Ins(1,4,5)P_3, and suggests that this type of behaviour is an intrinsic property of the Ins(1,4,5)P_3 receptor. The pattern of Ca^{2+} release was also similar to Ins(1,4,5)P_3 in that it was biphasic, with fast and slow release components.

A recent investigation into the kinetics of Ca^{2+} puffs evoked in *Xenopus* oocytes by different Ins(1,4,5)P_3 receptor agonists,[114] reported that adenophostin A was the first compound found to modulate the time-course of receptor activity during Ca^{2+} puffs. Unlike Ins(1,4,5)P_3, adenophostin A consistently failed to evoke repetitive Ca^{2+} spikes and oscillatory Ca^{2+}-activated Cl^- currents. There was also an absence of abrupt, regenerative Ca^{2+} release in response to gradually increasing concentrations of adenophostin A. In addition, Ca^{2+} release through the Ins(1,4,5)P_3 receptor evoked by adenophostin A was described as discrete Ca^{2+} puffs or as being more continuous than that invoked by Ins(1,4,5)P_3, without resolvable elementary events.

Several groups have investigated the ability of adenophostin A to release Ca^{2+} from intracellular stores, to activate influx Ca^{2+} entry and to activate calcium-release activated calcium currents (I_{CRAC}).[115-119] In general adenophostin A has been shown to

behave in a similar way to Ins(1,4,5)P_3 and activate I_{CRAC} as a consequence of release of Ca^{2+} release from intracellular stores, rather than directly opening SOCs., although the kinetics of Ca^{2+} release exhibited by adenophostin A were found to be somewhat different to those of Ins(1,4,5)P_3. In addition low concentrations of adenophostin A were shown to stimulate Ca^{2+} influx without stimulating a large Ca^{2+} release.[115] Further investigations with adenophostin A and Ins(1,4,5)P_3 by Gregory *et al.*[119] proposed that there is a functionally distinct subregion of the endoplasmic reticulum with a different apparent affinity for, or accessibility to, Ins(1,4,5)P_3 that is involved in the activation of SOCs in hepatocytes.

An interesting investigation by Bird *et al.*[120] examined the spatial features of Ca^{2+} signalling in *Xenopus laevis* oocytes using different concentrations of *myo*-inositol 2,4,5-trisphosphate, a metabolically stable Ins(1,4,5)P_3 analogue, and adenophostin A (also metabolically stable). They reported that when extremely low concentrations of adenophostin A were introduced into the oocytes (~10 pM), stable regions of Ca^{2+} release were observed, that did not expand to peripheral regions. When the Ca^{2+} release was restricted to central regions, compartmentalised Ca^{2+} oscillations were sometimes observed. Restoration of extracellular Ca^{2+} caused a rise in cytoplasmic Ca^{2+} restricted to the region of adenophostin A-induced Ca^{2+} mobilisation. These observations led to the suggestion that adenophostin A might be a useful tool in the examination of Ca^{2+} signalling processes under spatially restricted conditions, which may in turn lead to insights into mechanisms of intracellular Ca^{2+} oscillations and capacitative Ca^{2+} entry.

Another use for the adenophostins has been demonstrated by Sato *et al.*,[121] who found that a single injection of adenophostin A into mouse oocytes produced Ca^{2+} oscillations lasting for at least 3 hours similar to those observed at fertilisation. Simultaneous injection of adenophostin A and a round spermatid resulted in fertilisation of over half of the oocytes. From these experiments it was concluded that adenophostin A might be useful in parthogenetic oocyte activation in animal reproduction, in particular it may be applicable to assisted conception therapy for patients with defective spermatogenesis.

Structure

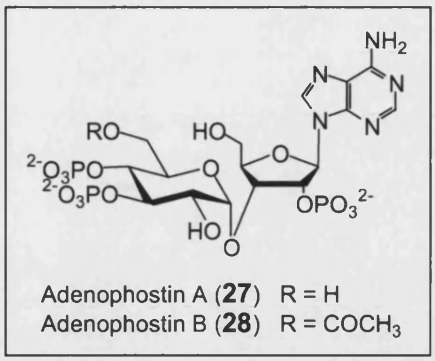
The structures of adenophostin A and B (Figure **1.15**) were elucidated by Takahashi *et al.*[122] shortly after their discovery. Purified and separated by preparative

HPLC, 30mg of adenophostin A and 100mg of adenophostin B were isolated from six hundred and eighty litres of cultured broth.[112] The molecular formula of adenophostin A and B were determined from elemental analysis and HRFAB-MS spectra. UV spectra indicated the presence of an *N*-9 substituted moiety, and this was confirmed by signals in both the ^{1}H NMR and ^{13}C NMR spectra which also indicated the presence of an adenine motif. In addition there was a fragment ion at *m/z* 136 in the FAB-MS spectrum which corresponded to an adenine structure. The presence of an adenine structure was further corroborated by deamination of adenophostin A by treatment with $NaNO_2$ in acetic acid to give the inosine derivative which exhibited a typical UV absorption maximum at 248 nm.

Adenophostin B was identified as the acetyl derivative of adenophostin A from the ^{1}H NMR spectrum in which there was an additional acetyl signal. Adenophostin B was treated with aqueous NaOH solution to give a compound identical to adenophostin A. Comparison of the ^{1}H NMR spectra of adenophostin A and B determined that adenophostin B was substituted at position 6", since the 6"-CH_2 signal in the spectrum of adenophostin B was observed 0.5~0.7 ppm further down-field than in the spectrum of adenophostin A.

Further NMR correlation experiments on adenophostin B established that part of the adenophostins structure was made up of a combination of a five carbon and six carbon sequence. Alkaline phosphatase was utilised to give phosphorus free product, and after modification of conditions, a bisphosphorylated structure. ^{31}P and ^{1}H NMR of this product determined that the free hydroxyl was at the 2'-position.

The dephosphorylated material treated with 10% HCl in MeOH and the products trimethylsilylated. GC-MS allowed the identification of these trimethylsilyl derivatives as adenine, ribose and glucose. Thus the adenophostins were assigned as α-glucosyl adenosine derivatives, with the position of the glucosyl linkage being determined as the 3'-position. Finally the positions of the other two phosphate groups were deduced to be at the 3" and 4" positions of the glucopyranoside ring using ^{1}H-^{31}P NMR correlation spectra.



Adenophostin A (**27**) R = H
Adenophostin B (**28**) R = $COCH_3$

Figure **1.15**: The structure of adenophostin A and adenophostin B.

Even though the structures of the adenophostins are quite dissimilar to Ins(1,4,5)P_3, as previously discussed they exhibit a marked increase in Ca^{2+} mobilising ability. We now have a reasonable understanding of the structure-activity relationships of Ins(1,4,5)P_3, and on comparison of its structure with the adenophostins there is clear similarity between the adenophostin 3",4"-bisphosphate/-2'-hydroxy motif and the Ins(1,4,5)P_3 4,5-bisphosphate/-6-hydroxy motif central to receptor binding and Ca^{2+} mobilisation. These common characteristics, while giving an indication to potency at the Ins(1,4,5)P_3 receptor, do not explain the exceptional ability of the adenophostins to mobilise Ca^{2+} through the Ins(1,4,5)P_3 receptor. Thus, further information was required about the structure-activity relationships of the adenophostins. Although it is known that the deletion of the 2' phosphate reduces the affinity of adenophostin A for the Ins(1,4,5)P_3 receptor by a factor of almost 2000,[122] little is known about the features of the adenosine moiety that enhance activity.

1.9 Aims of this work

Some of the basic structure-activity relationships of adenophostin A and B have been alluded to in the preceding discussion, but a more complete understanding is clearly required to aid the design of more effective pharmacological modulators of the Ins(1,4,5)P_3 receptor, possibly even antagonists.

It has already been stated that the synthesis of Ins(1,4,5)P_3 analogues from chiral carbohydrate starting materials is an attractive prospect. Thus the discovery of the adenophostins provides an excellent opportunity for the design and synthesis of carbohydrate-based compounds active at the Ins(1,4,5)P_3 receptor.

To investigate the exciting structure-activity relationships of the adenophostins the following aims were identified:

- An efficient synthesis of adenophostin A.
- The synthesis of minimal structure analogues lacking the adenine base of adenophostin A.
- The synthesis of sugar modified adenophostin A analogues, with modification at the 2"-hydroxyl and the 5"-hydroxymethyl.
- The synthesis of base modified adenophostin A analogues from a common disaccharide intermediate.
- Biological evaluation of synthesised analogues for Ca^{2+} release and binding at the Ins(1,4,5)P_3 receptor.

Chapter two

Synthesis of Adenophostin A and its biological evaluation

2 Synthesis of Adenophostin A and its biological evaluation

2.1 Introduction

To confirm the structural assignment given for the adenophostins by Takahashi *et al.*[122] their total synthesis was vital. It was deduced that the basic backbone of their structure consisted of adenosine connected through its 3'-position to a glucopyranose ring *via* an α-glycosidic linkage. In addition they were found to be trisphosphates, with a vicinal 3,4-bisphosphate on the glucopyranose ring, and a further phosphate placed at the 2'-position of the adenosine portion of their structure. Adenophostin B was also found to be the 6"-acetate of adenophostin A.

It was decided that the synthesis of adenophostin A would be attempted and that the synthetic strategy would be based around the glycosidation of a regioselectively protected adenosine acceptor with a suitably protected glucosyl donor (Figure **2.1**).

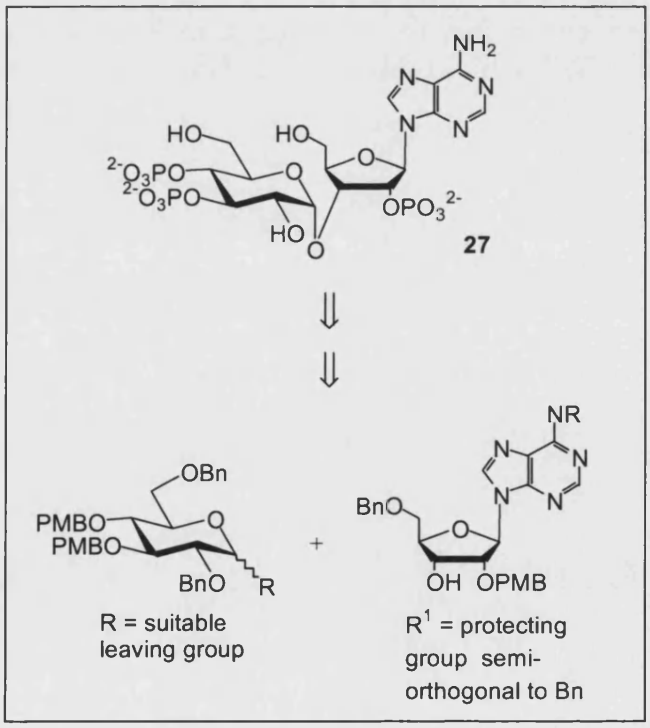


Figure **2.1**: Retrosynthetic analysis of adenophostin A.

2.2 Synthesis of adenosine-based glycosyl acceptors

As discussed in chapter 2 adenosine presents a number of problems in regioselective protection with orthogonal groups. Four differently protected adenosine acceptors were made, one of which was found to be the most suitable for coupling to the glycosyl donor. These will now be discussed individually.

2.2.1 N^6-Benzoyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-monomethoxytrityl adenosine (35)

2.2.1.1 Proposed route

The stimulus for this protecting group strategy came from a paper describing the regioselective protection of the 2'-hydroxyl of adenosine with a *p*-methoxybenzyl ether.[123] The surprising selectivity for the 2'-secondary hydroxyl in this reaction was attributed to a favourable π-stacking interaction between the adenine purine ring and the phenyl ring of the *p*-methoxybenzyl group. Since the 3,4-vicinal diol of the glucosyl donor would be protected as *p*-methoxybenzyl ethers, it followed that protection of the adenosine 2'-position should be with the same group. Future selective deprotection of the *p*-methoxybenzyl groups would then lead to a triol suitable for phosphorylation to give adenophostin A.

The most widely (and routinely) used protecting group at the N^6-position of adenine is the benzoyl amide and it was deemed suitable for this role in the proposed adenosine acceptor. It is easily introduced with benzoyl chloride in pyridine, and easily removed with methanolic ammonia or sodium methoxide. The synthesis of this intermediate has been reported using trimethylsilyl groups to transiently protect free ribosyl hydroxyl groups[124] followed by benzoylation and subsequent cleavage of the silyl ethers with concentrated aqueous ammonia. However, in this synthetic route alkaline cleavage of unwanted esters was employed, since it has also been reported that any undesired esters (which in this case would be formed at the adenosine 5'- and 3'-positions) are easily cleaved selectively by short exposure to base leaving the relatively stable N^6-benzoyl amide in place.[125] This adenine-based amide remains intact because the ionisation of an amide group in an aromatic system which occurs at alkaline pH extends the resonating system and thus stabilises the acyl on the amino function.

Finally it was decided that the 5'-hydroxyl should be protected as a benzyl ether as this would be stable to the conditions required for the cleavage of the *p*-methoxybenzyl ether and the amide at positions 2' and N^6 respectively. It was reasoned that this primary hydroxyl would be alkylated in preference to the 3'-hydroxyl to give the desired acceptor for coupling.

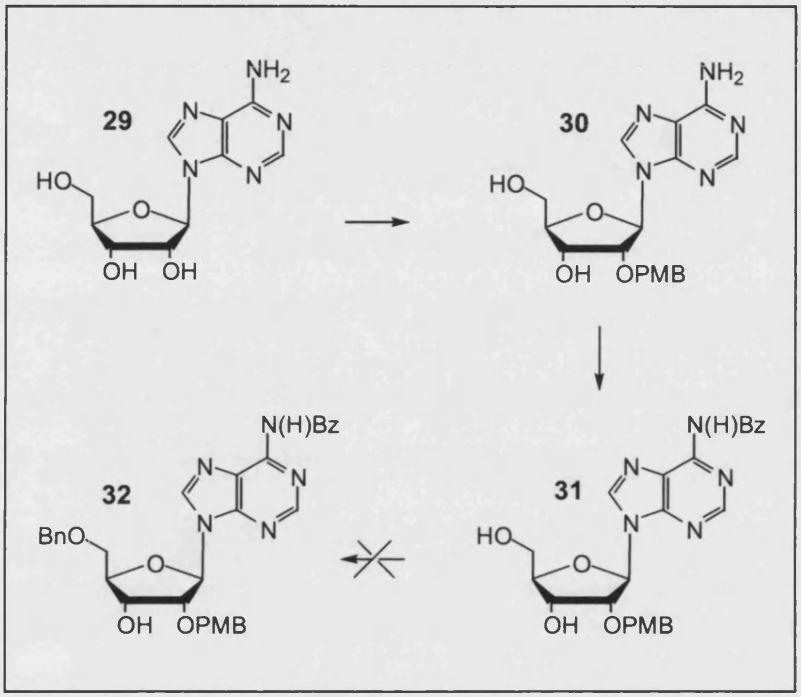


Figure **2.2**: Proposed route to adenosine based acceptor **32**.

2.2.2.2 Discussion of synthetic work

Adenosine (**29**) was converted into the 2'-*O*-*p*-methoxybenzyl derivative **30** with melting point 153°C (lit.,[123] 155–156°C) by a method adapted from the literature procedure. The resulting product's ^{1}H NMR spectrum corresponded with the published data and substitution at the 2'-position could easily be ascertained from the ^{1}H-^{1}H COSY NMR spectrum in D_6 DMSO. Couplings of the 3'- and 5'-hydroxyl protons to methine and methylene protons respectively was apparent, but no such signal or coupling was observed for a 2'-hydroxyl. The yield was somewhat lower than that published (32%, *cf.* 65%) and this was mainly attributed to the use of the commercially available *p*-methoxybenzyl chloride instead of the less stable bromide. The former reagent is less reactive and therefore required a slightly higher temperature for reaction to take place,

which in turn led to decreased selectivity for the 2'-position and incomplete conversion of starting material into product.

Attention then turned to protection of the N^6-position. Using a method adapted from Schaller, *et al.*,[125] 2'-*O*-*p*-methoxybenzyl adenosine was converted into the 3'-*O*-,5'-*O*-,N^6, N^6-tetrabenzoyl derivative. Careful alkaline hydrolysis of this intermediate with 1M aqueous sodium hydroxide solution in ethanol furnished the desired N^6-benzoyl product (**31**). Inspection of the ^{1}H NMR spectrum soon confirmed the presence of the desired amide at N^6 with the appearance of a very deshielded proton at δ_H 11.23, corresponding to the amide proton which exhibited D_2O exchange. The rest of the ^{1}H NMR spectrum was also found to be in agreement with that reported in the literature.

Finally, benzylation of the 5'-hydroxyl of N^6-benzoyl-2'-*O*-*p*-methoxybenzyl adenosine was attempted using sodium hydride and benzyl bromide in DMF. The reaction yielded two unexpected products, one major (66%), and one minor (30%). On examination of their ^{1}H NMR spectra (solvent D_6 DMSO) it was apparent that the pseudo triplet of the 5' hydroxyl of the adenosine ribose ring was still present, yet signals in the aromatic region indicated alkylation with a benzyl group. On closer examination it became apparent that neither ^{1}H NMR spectrum had signals from the N^6 benzoyl amide proton, which suggested that alkylation had occurred at this amide. Furthermore the ^{13}C NMR spectra indicated the appearance of Ph*C*H$_2$ signals at 51–53ppm, typical of alkylation at nitrogen, as opposed to oxygen. Finally UV analysis revealed two very different spectra. The minor product gave a similar spectrum to the starting material, with a small bathochromic shift, whereas the major product seemed to exhibit a loss of conjugation in the purine ring, with a consequent loss of the absorption maxima exhibited by the starting material and minor product. This would suggest that the major product was alkylated at one of the nitrogens in the purine ring system, probably N^1 (**33**), and the minor product was alkylated at N^6 (**34**).

The explanation for the formation of these two products lies in the relative pKa values of the N^6amide proton and the 5'-hydroxyl proton. Ionisation of an amide group in an aromatic system occurs at alkaline pH which extends the resonating system through the adenine amine and in this case it led to alkylation at either N^1 or N^6 (see Figure **2.3**) instead of the 5'-hydroxyl.

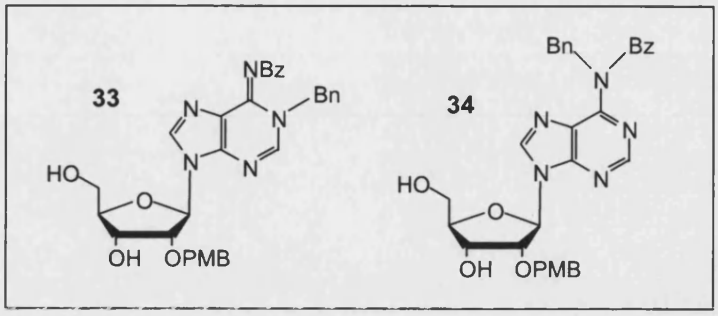


Figure **2.3**: The isolated alkylation products of **31**.

Further attempts to selectively benzylate the adenosine 5'-position of **31** and **30** with reduced temperature,[126] bis(tributyltin) oxide, silver oxide[127] and benzyl trichloroacetimidate[128] all failed. At this point it was decided that a monomethoxytrityl group was the best protecting group for protection of the 5'-hydroxyl. Treatment of **31** with 1.5 equivalents of monomethoxytrityl chloride in pyridine gave the 5'-*O*-monomethoxytrityl derivative in high yield. (While this work was in progress the first synthesis of adenophostin A was published using this adenosine acceptor[129]). Glycosidation reactions with this acceptor however were unsuccessful and will be discussed in more detail later, therefore the synthesis of another acceptor was begun.

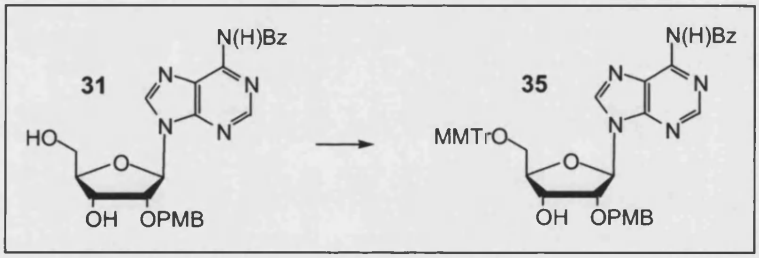


Figure **2.4**: Revised 5'-*O*-protection of **31**.

2.2.2 5'-*O*-Benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine

2.2.2.1 Synthesis of 2',3'-*O*-*p*-methoxybenzylidene adenosine (36)

The main shortcoming of the above adenosine-based acceptor was the steric bulk of the 5'-*O*-monomethoxytrityl ether. Benzyl protection at this position was thought desirable but not achieved, so a different approach was adopted. In the synthesis of the methyl β-D-ribofuranoside based acceptor of ribophostin the 2-*O*-*p*-methoxybenzyl ether

was introduced by reductive cleavage of a 2,3-*O*-*p*-methoxybenzylidene acetal. The principal advantage of this approach was that the 2,3-*O*-*p*-methoxybenzylidene protected the 3-hydroxyl while a benzyl ether was introduced at the 5-position.

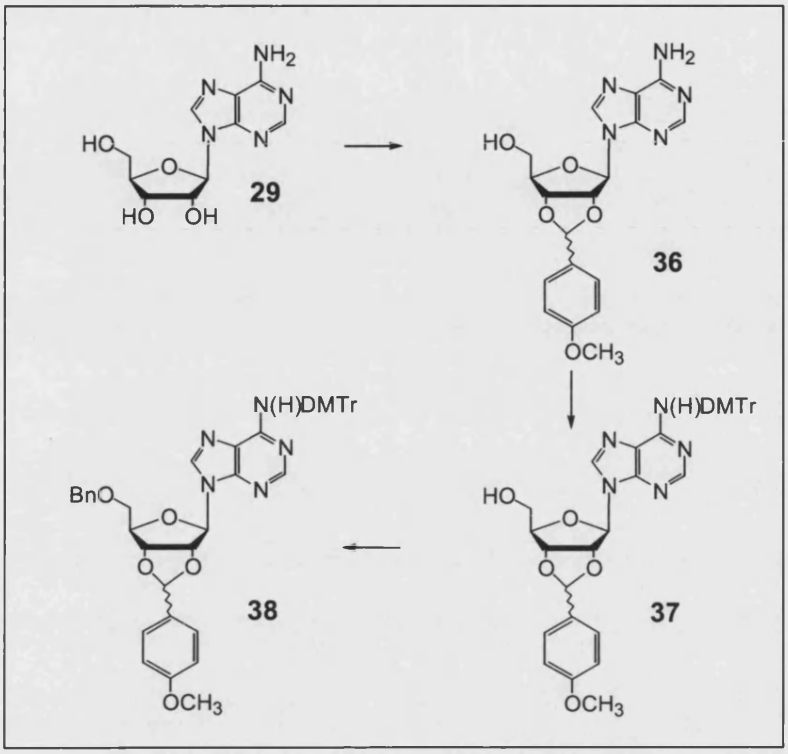


Figure **2.5**: Route to **38**.

Similar protection of the 2' and 3'-positions of adenosine would therefore enable alkylation at the 5'-position once N^6 had been adequately protected. A search of the literature found three slightly different methods for the preparation of 2',3'-*O*-*p*-methoxybenzylidene nucleosides. Both 2',3'-*O*-*p*-methoxybenzylidene uridine[130] and 2',3'-*O*-*p*-methoxybenzylidene inosine[131] were prepared by essentially the same method first published by Michelson and Todd[132] for the synthesis of 2',3'-*O*-benzylidene adenosine, where starting material was stirred with a mixture of the Lewis acid zinc chloride and *p*-methoxybenzaldehyde. The desired products were formed by nucleophilic attack by the 2'- and 3'-hydroxyl groups on the *p*-methoxybenzaldehyde-zinc chloride complex. It must be noted here that although Michelson and Todd reported their acetal as the 3',5'-*O*-benzylidene, they had in fact made the 2',3'-*O*-benzylidene, and subsequently in the literature it is referred to as such. It has been established that the *trans* configuration of the 3- and 5-hydroxyls in a ribofuranose ring discourages acetal formation, while the *cis* relationship of the 2,3 *cis*-diol favours it.

In the second method 2',3'-*O*-*p*-methoxybenzylidene adenosine[133] and 2',3'-*O*-*p*-methoxybenzylidene 8-azaadenosine[134] were prepared from a mixture of *p*-methoxybenzaldehyde, triethyl orthoformate and hydrogen chloride solution in dioxane. The mechanism for this reaction is different to that described above and was investigated in the above paper describing the synthesis of 2',3'-*O*-*p*-methoxybenzylidene adenosine. The authors discuss two roles for triethyl orthoformate; firstly as a dehydrating agent, and secondly under acidic reaction conditions it activates the *p*-methoxybenzaldehyde as a diethyl acetal which reacts by acetal exchange.

Finally a slight modification of the previous method with *p*TSA instead of hydrogen chloride solution was reported by Morley and Hogenkamp.[135] The use of tosic acid as the proton source led to shorter reaction time and greater yields without the rigorous drying of reactants.

Formation of a 2,3-*O*-acetal in a ribofuranoside (such as that found in adenosine) by reaction with an aldehyde generates a new stereogenic centre. The configuration of the two benzylidene acetal diastereoisomers formed at this position has been investigated and unambiguously assigned by using NOE NMR spectroscopy.[136] The *exo* isomer acetal H lies underneath the ribosyl ring, and therefore irradiation at the H-4' frequency led to an enhancement of this acetal H signal (Figure **2.6**). The other diastereoisomer (*endo*) exhibited no such enhancement. This assignment confirms an early report outlining the preparation of both benzylidene diastereoisomers by variation of reaction temperature.[137] Low temperature yielded one isomer, while high temperature yielded another; these were assigned as the *endo* and *exo* diastereoisomers and designated the kinetic and thermodynamic acetals respectively.

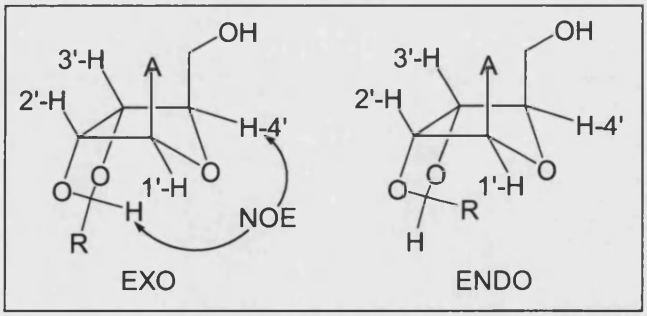


Figure **2.6**: Diagram of the acetal diastereoisomers of 2',3'-*O*-*p*-methoxybenzylidene adenosine.

The required 2',3'-*O*-*p*-methoxybenzylidene adenosine was initially prepared by a modification of the Michelson and Todd method where the *p*-methoxybenzaldehyde-zinc

chloride complex was preformed before the addition of dry adenosine in order to maximise the yield.[138] Following the initial work up most of the product was isolated by precipitation from *p*-methoxybenzaldehyde and found to be a single diastereoisomer by ^{1}H NMR. After addition of diisopropyl ether the remaining product was isolated by precipitation and was found to be a mixture of diastereoisomers in a ratio of 2:1 as indicated by the ^{1}H NMR integrals of the *p*-methoxybenzylidene C*H* signals (combined yield of 66%). Their configurations were assigned by comparison of their ^{1}H NMR data to that published for the benzylidene equivalents. The major isomer was assigned as *endo*, the kinetic product. This reaction was repeated with elevated temperature to explore the acetal-equilibrium associated with *p*-methoxybenzylidene diastereoisomers. The resulting product was an inseparable diastereoisomeric mixture in a ratio of 3:2 *exo* : *endo* as indicated by the ^{1}H NMR integrals of the *p*-methoxybenzylidene C*H* signals. As this result was contrary to that reported by Baggett *et al.*[137] for the benzylidene acetal, the experiment was repeated using benzaldehyde. In the author's hands the *endo* isomer was prepared successfully, but the high temperature preparation lead to a mixture of diastereoisomers in a ratio of 3:2 *exo* : *endo* as indicated by the ^{1}H NMR integrals of the benzylidene C*H* signals. So, it is probable that the ratio of 3:2 *exo* : *endo* is the point at which equilibrium is reached with no further equilibration in favour of the *exo* product. This was substantiated by taking the *p*-methoxybenzylidene *endo* diastereoisomer and exposing it to the same reaction conditions at elevated temperature. The isolated product from this reaction was again a mixture of diastereoisomers in a ratio of 3:2 *exo* : *endo*.

The reported method of Morley and Hogenkamp[135] was also employed in an effort to improve the yield of the 2',3'-*O*-*p*-methoxybenzylidene derivative and to establish whether this method furnished a similar diastereoisomeric mixture to that of Michelson and Todd. The product was isolated in improved yield and the ^{1}H NMR spectrum (Figure **2.7**) displayed the same mixture of diastereoisomers (in a ratio of 3:2 *exo* : *endo*) as that obtained from the elevated temperature experiments above.

Finally a method to prepare 2',3'-*O*-*p*-methoxybenzylidene adenosine by direct acetal exchange was attempted,[139;140] since it had proved successful for the preparation of methyl 2,3-*O*-*p*-methoxybenzylidene-β-D-ribofuranoside in the synthesis of ribophostin.[141] *p*-Methoxybenzaldehyde dimethyl acetal, tosic acid and adenosine in DMF were heated to 50°C in a flask fitted with an air condenser to remove any liberated methanol. No product was detected by TLC so this method was abandoned.

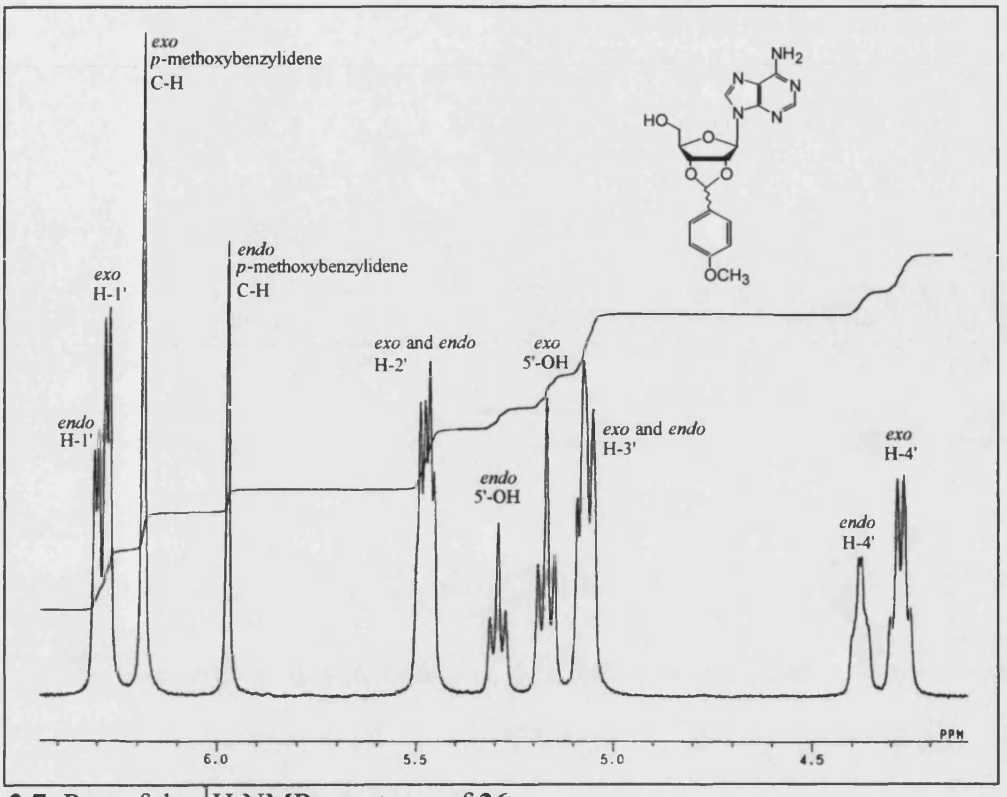


Figure **2.7**: Part of the ^{1}H NMR spectrum of **36**.

For reasons that will be outlined in future discussion *endo* 2',3'-*O*-*p*-methoxybenzylidene adenosine and the diastereoisomeric mixture were both carried forward and used in subsequent reactions.

2.2.2.2 Selective protection of N^6

Alkylation of the 5'-hydroxyl could not take place until N^6 had been protected. Work previously discussed indicated that the benzoyl group was unsuitable for this purpose and so a protecting group was sought to block N^6 totally and which would be stable in the strongly basic conditions required for alkylation. After searching the literature it was found that the trityl group was sometimes used to protect N^6,[142] and references therein, although it was found that tritylation at N^6 involved forcing conditions and long reaction times. In 1968 Smith *et al.*[130] reported two new modifications of the trityl group. They substituted either one or two of the trityl phenyl rings with a *p*-methoxyphenyl ring. For each *p*-methoxyphenyl added they found an approximately 10-fold increase in the rate of acid hydrolysis. Each substitution with a *p*-

methoxyphenyl ring is also associated with an increase in reactivity of the corresponding chloride; consequently the reaction conditions needed for its introduction are much milder, even at N^6. Thus it is the presence of added *p*-methoxyphenyl groups that confer both increased reactivity of their respective chlorides and increased acid sensitivity by stabilising the trityl carbocation.

The properties of the dimethoxytrityl group therefore seemed ideal for the protecting group needed to mask N^6 in the manner described above. However one potential problem remained, i.e. the protection of N^6 in the presence of a free 5'-hydroxyl. A transient protecting group was needed. Use of the trimethylsilyl group to transiently protect free ribosyl hydroxyls is well established[124;143] to facilitate selective N^6-benzoylation in a simple one-pot procedure. Addition of chlorotrimethysilane to a solution of the starting material in dry pyridine prior to the addition of benzoyl chloride results in the desired N^6 benzoylation, and the trimethylsilyl ester(s) are later easily cleaved by adding concentrated aqueous ammonia solution. To the best of the authors knowledge use of trimethylsilyl transient protection had not been attempted in conjunction with N^6-dimethoxytritylation and was therefore investigated. Thus a mixture of 2',3'-*O*-*p*-methoxybenzylidene adenosine with chlorotrimethylsilane in dry pyridine was stirred for 2 hours before the addition of dimethoxytrityl chloride, and the resulting reaction mixture was left overnight. Subsequent addition of concentrated aqueous ammonia solution cleaved the trimethylsilyl group to furnish the desired N^6 protected material in 97% yield after column chromatography.

The ^1H NMR spectrum of **37** in d_6 DMSO exhibited a D_2O exchangeable triplet at δ_H 5.22 typical of the 5'-hydroxyl signal (Figure **2.8**), indicating that the 5'-hydroxyl was still unprotected.

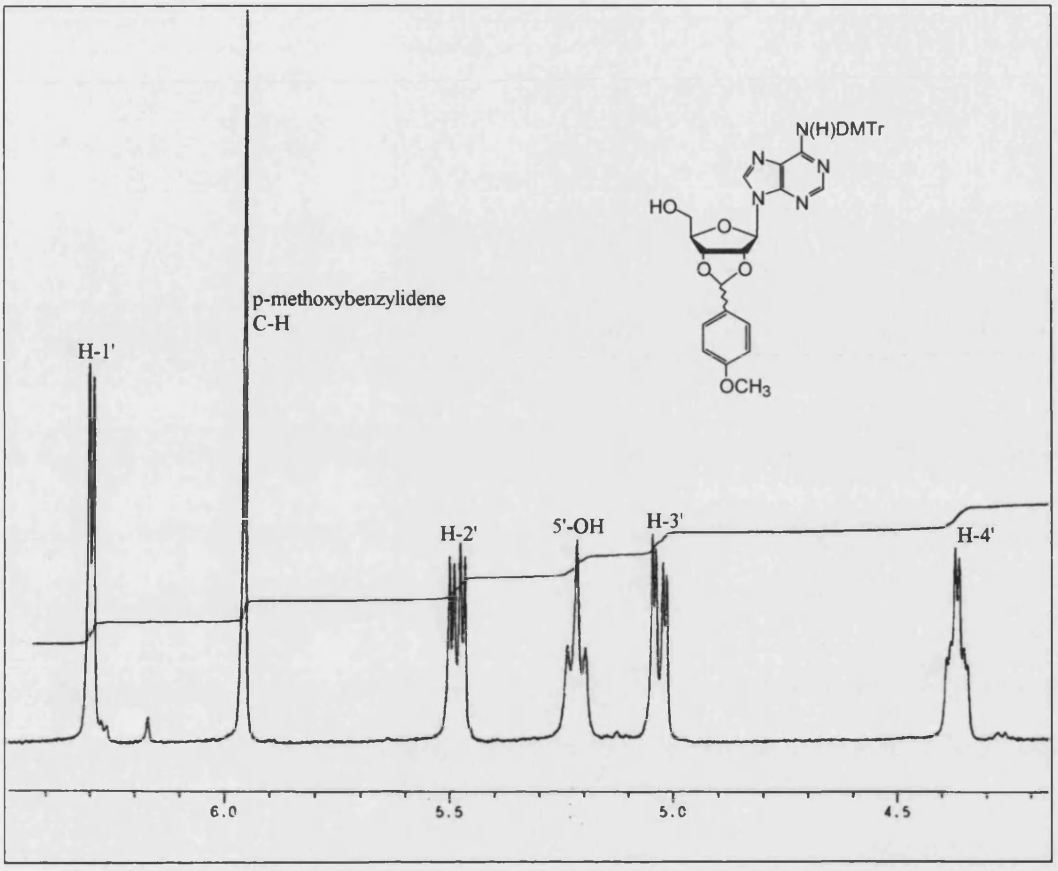


Figure **2.8**: Part of the ^{1}H NMR spectrum of **37** indicating the free 5'-hydroxyl signal.

2.2.2.3 5'-*O*-Alkylation

Alkylation of the 5'-hydroxyl group proved more difficult than expected. Treatment of N^6-dimethoxytrityl-2',3'-*O*-*p*-methoxybenzylidene adenosine with sodium hydride and benzyl bromide in DMF at room temperature in the usual manner gave no discernable product by TLC. Heating to 70°C with a large excess of alkylating reagents gave a poor yield (39%). A search of the literature revealed a benzylation procedure for adenosine derivatives by refluxing with benzyl chloride and potassium hydroxide in a mixture of dioxane and benzene[144]for 15 minutes. N^6-Dimethoxytrityl-2',3'-*O*-*p*-methoxybenzylidene adenosine was subjected to these conditions and the desired material (**38**) isolated in 95% yield after column chromatography. The ^{13}C NMR spectrum displayed a CH_2 signal at δ_C 73.45 in the region for CH_2O resonances thus confirming alkylation at the 5'-hydroxyl with a benzyl ether. This was substantiated by the C-5' signal being deshielded to δ_C 70.10 (the α-effect of alkylation).

2.2.2.4 Reductive cleavage of the 2',3'-*O*-*p*-methoxybenzylidene acetal of 38

Investigations into regioselective cleavage of *p*-methoxybenzylidene acetals to *p*-methoxybenzyl ethers have resulted in conditions that fall into two groups. $LiAlH_4/AlCl_3$, $BH_3{\cdot}NMe_3/AlCl_3$, BH_3, THF, heat, or $NaBH_3CN$/TMSCl, CH_3CN all result in cleavage at the least hindered side of the acetal, giving the more hindered ether, whereas treatment with $NaBH_3CN$/HCl, $NaBH_3CN$/TFA/DMF and DIBAL-H results in the formation of an ether at the least hindered hydroxyl.[145]

Since the conditions above mainly relate to ether formation from six-membered rings acetals (1,3-dioxanes) as opposed to five membered ring acetals (1,3-dioxolanes) as found in **38**, it was difficult to predict the direction of its cleavage with any of the preceding reagents. Nevertheless successful cleavage of a 2,3-*O*-*p*-methoxybenzylidene derivative of ribose (a 3:2 diastereoisomeric mixture) has been reported with DIBAL-H to yield predominantly the desired 2'-*O*-ether.[141] Similar treatment of the inseparable 3:2 *exo* : *endo* diastereomeric mixture of **38** with DIBAL-H in DCM at 0°C met with little success, and only traces of the desired product were isolated. Further experimentation established that the correct temperature was crucial; if the temperature was too high there was complete cleavage of the acetal to the diol, whereas if the temperature was too low there was no reaction at all. Thus addition of DIBAL-H at –78°C followed by slow warming to *ca.* –25°C over 2 hours gave reductive acetal cleavage in high yields. The product was found to be a mixture of regioisomers in a 3:2 ratio with the desired 2'-*O*-*p*-methoxybenzyl ether (**40**) predominating. This mixture was inseparable so it was acetylated with acetic anhydride in pyridine under standard conditions (yield over two steps 93%). It was then possible to separate the two products by careful flash column chromatography. Not only did the introduction of an acetyl group aid purification of the regioisomers, but it also made their identification easier. In both ^1H NMR spectra of **41** (yield 38%) and **42** (yield 55%) there were deshielded protons corresponding to the respective acylated positions. Once identified, the 3'-*O*-acetyl derivative was then treated with methanolic ammonia to furnish the desired glycosyl acceptor in quantitative yield.

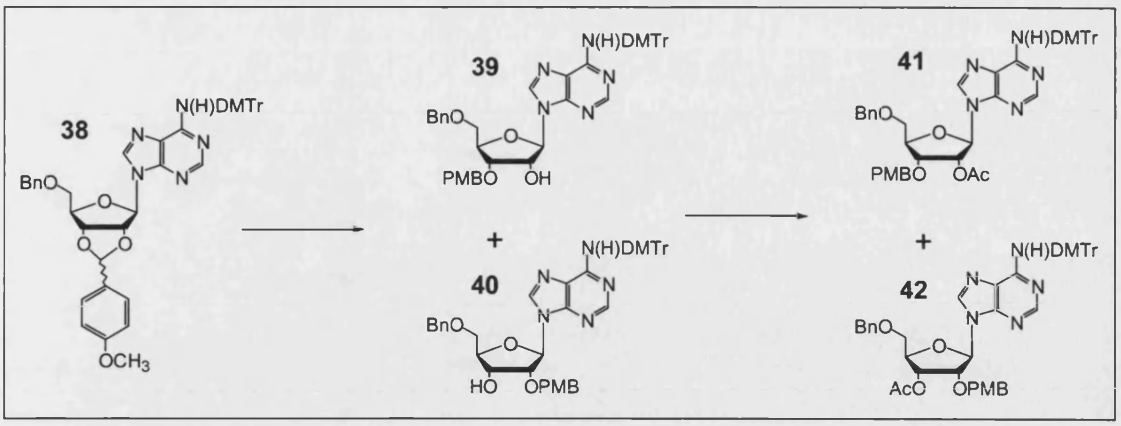


Figure **2.9**: Reductive cleavage of **38** with DIBAL-H and acetylation of the product mixture.

This reduction was investigated further to ascertain whether its regioselectivity was dependent on the starting diastereoisomer configuration. Treatment of the *endo* diastereoisomer of **38** with DIBAL-H resulted in conversion mainly to the undesired 3'-*O*-*p*-methoxybenzyl ether.

Discussion in the literature is in general agreement that reductive cleavage of an acetal to an ether with DIBAL-H as reducing agent is highly dependent on the stereochemical environment of the molecule.[146;147] DIBAL-H is thought to coordinate to the least hindered acetal oxygen with subsequent acetal cleavage occurring at that position. Therefore DIBAL-H is an ingenious reagent in that it acts both as the Lewis acid and the hydride donor. From the regioselectivity of the reductions of the *endo* and *endo*/*exo* mixture of diastereoisomers of **38** this would suggest that DIBAL-H coordinates mainly with the 2'- and 3'-oxygens of the *endo* and *exo* diastereoisomers respectively to give the corresponding 3'-*O*- and 2'-*O*-*p*-methoxybenzyl ethers as shown in Figure **2.10**.

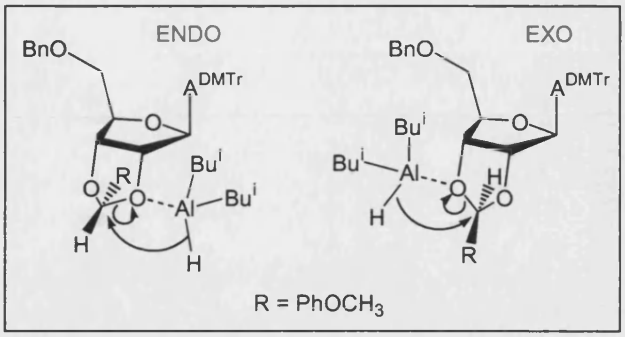


Figure **2.10**: Coordination of DIBAL-H to *endo* and *exo* diastereoisomers of **38**. (A = adenine)

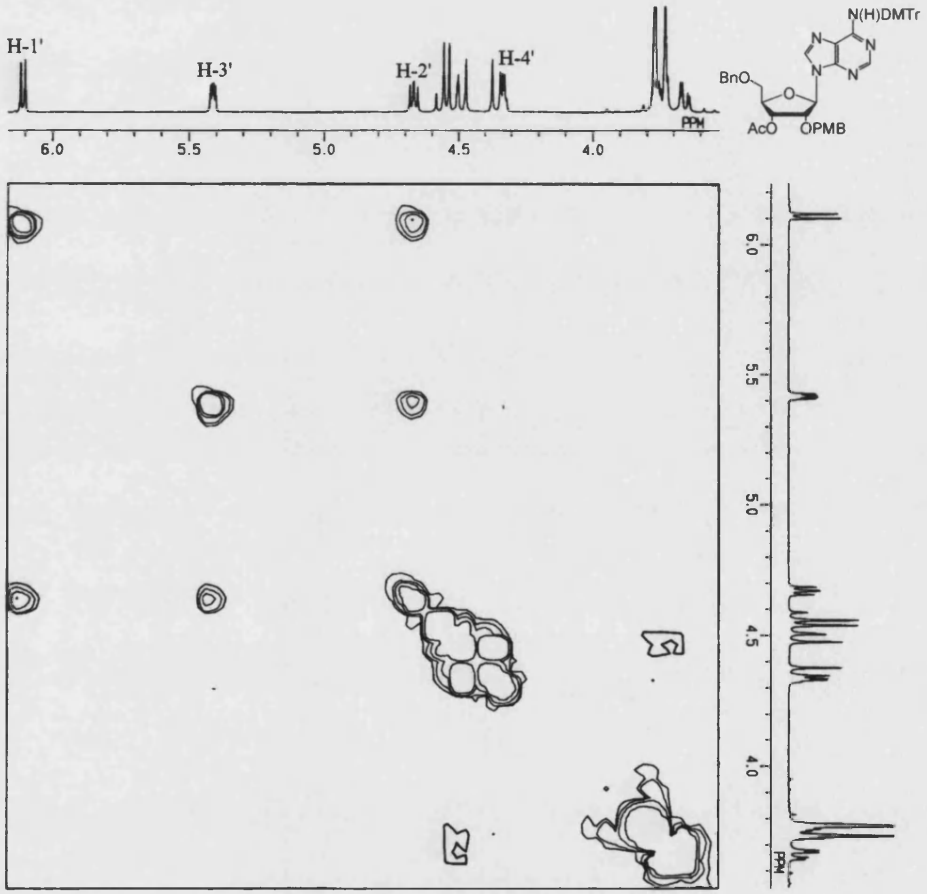


Figure **2.11**: Part of the ^{1}H-^{1}H COSY spectrum of **42** indicating the acylation of the 3'-hydroxyl.

Attempts at the acetal reduction with other literature reagents mentioned earlier had little success and none gave the desired regioisomer in anything more than a trace amount as judged by TLC.

2.2.3 Synthesis of other potential acceptors

2.2.3.1 N^6,N^6-Dibenzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine (46) and N^6-benzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine (32)

While suitable conditions to reductively cleave the *p*-methoxybenzylidene of **38** to the desired regioisomer were being sought, this intermediate was converted into two other acceptors, one of which was the initial target acceptor that could not be made by the first route.

2.2.3.2 Proposed route

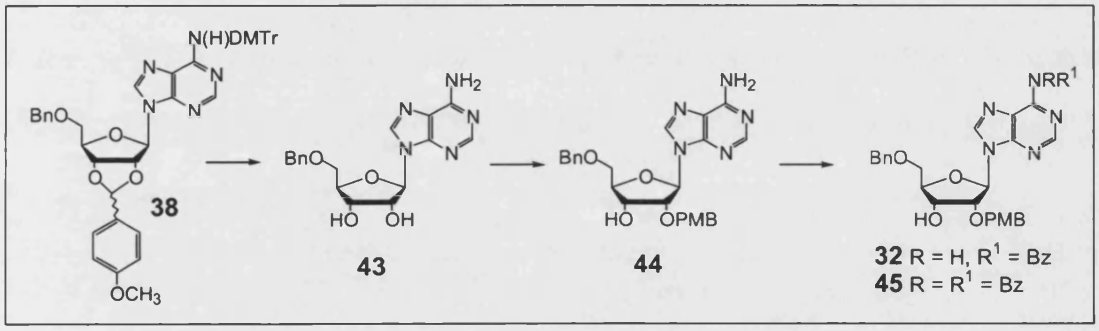


Figure **2.12**: Proposed route to 32 and 45.

It was quickly realised that if suitable reductive cleavage conditions were not found intermediate **38** could be used to make acceptors by an alternative route. *p*-Methoxybenzylidene and dimethoxytrityl groups are both susceptible to acid hydrolysis, in fact a *p*-methoxybenzylidene is about ten times more labile to acid than the more frequently used isopropylidene and benzylidene acetals.[130] After treatment with acid it was proposed that regioselective *p*-methoxybenzyl ether formation at the 2'-hydroxyl with *p*-methoxybenzyl bromide followed by either mono- or di- N^6 benzoylation would furnish two new glycosyl acceptors.

2.2.3.3 Discussion of synthetic work

Cleavage of the dimethoxytrityl and *p*-methoxybenzylidene groups of **38** was achieved easily with 80% aqueous acetic acid at 100°C for 45 minutes in good yield to give **43** as a fine white powder. Attempts at selective alkylation of **43** with *p*-

methoxybenzyl chloride failed so *p*-methoxybenzyl bromide was freshly prepared by the literature method of Uwaydah *et al.*[148] Careful addition of *p*-methoxybenzyl bromide in DMF to a solution of the pre-formed alkoxide in DMF gave selective *p*-methoxybenzyl ether formation at the 2'-hydroxyl. The position of this ether was confirmed by acetylation of **44** with acetic anhydride. ^{1}H NMR revealed a deshielded doublet of doublets at δ_H 5.46 with coupling constants (*J* 5.1 Hz, 2.4 Hz) corresponding to acetylated H-3'.

Initially the N^6,N^6-dibenzoyl derivative (**45**) was prepared[143] because it was thought that introduction of two benzoyl groups would enhance solubility in the glycosylation solvent. The free 3'-hydroxyl was transiently protected with chlorotrimethylsilane before acylation of N^6 with benzoyl chloride. Subsequent addition of water cleaved the trimethylsilyl group in less than 30 minutes to give the desired product.

Preliminary glycosylation experiments with **45** indicated that some reaction conditions caused loss of one benzoyl group. So the monobenzoyl derivative **32** was prepared, essentially by the same reaction conditions as above, except that concentrated aqueous ammonia was added of instead of water to cleave both the trimethylsilyl group and one of the N^6 benzoyl groups.

2.3 Synthesis of the donor

Initially it was thought that the trichloroacetimidate donor used in the synthesis of the minimal structure analogue ribophostin (discussed in chapter **3**), would be ideal for coupling to an adenosine-based acceptor, but after several attempts using this glycosylation methodology with two differently protected adenosine acceptors (**35** and **40**) only traces of product could be isolated. At first it was thought that the steric bulk of a monomethoxytrityl group at the 5'-position of the acceptor **35** was impeding the reaction, but attempts with acceptor **40**, in which the 5'-postion was protected with a sterically smaller benzyl group also failed. The low reactivity of the acceptor was probably responsible for the absence of any reaction in either of these attempted glycosidations. A different coupling methodology was therefore sought.

After a thorough search of the literature, phosphite glycosidation methodology appeared the most promising for this particular coupling (reviewed [149]). This

glycosidation method was chosen for a number of reasons. First, a phosphite donor had been used successfully by Corey and Wu[150] with a $ZnCl_2$-$AgClO_4$ promoter to glycosylate a sterically hindered alcohol that was part of an acid sensitive intermediate, in the total synthesis of paeoniflorin. They also noted that all other common glycosidation methods had failed to give the desired compound. This was encouraging for the present synthesis as it suggested that a phosphite donor could be used to glycosylate the relatively unreactive adenosine 3' secondary hydroxyl. Secondly, many model glycosidations using phosphite donors have been reported by Watanabe *et al.*[151;152] with good α-stereoselectivity, and good yields. These authors have also reported the successful use of phosphite chemistry in the synthesis of a glycosyl phosphatidylinositol, 2,6 di-*O*-α-D-mannopyranosylphosphatidyl-D-*myo*-inositol.[153] Finally, the dimethyl phosphite donor needed for the glycosylation reactions is easily prepared in quantitative yield from the glucopyranose **46** which was already in hand as an intermediate from the synthesis of ribophostin (see chapter **3**).

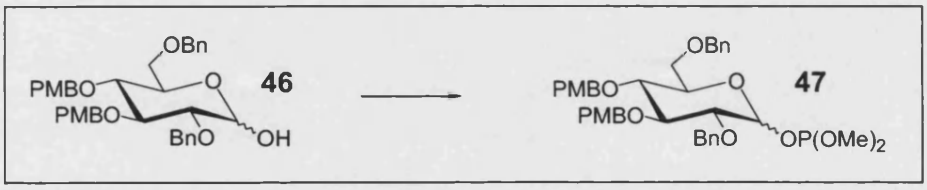


Figure **2.13**: Conversion of **46** into a dimethyl phosphite

Conversion of 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose (**46**) into its dimethyl phosphite (**47**) was achieved with bis(methoxy)(diethylamino)phosphine activated by tetrazole (Figure **2.13**) in a similar manner to phosphitylation with bis(benzyloxy) (diisopropylamino)phosphine and tetrazole employed in the synthesis of phosphates. ^{1}H NMR of the product indicated an anomeric mixture in a ratio of 1:1 as estimated from the integral ratio of the anomeric protons. The presence of an anomeric mixture of phosphites was confirmed by ^{31}P NMR with two resonances of equal integral in the phosphite region at δ_p 141.14 and 142.31.

2.4 Glycosidation and deprotection

Glycosidation proved the most difficult step in this route. As mentioned previously it was quickly realized from preliminary experiments with **35** or **40** as glycosyl acceptor and a trichloroacetimidate glycosyl donor that this methodology was unsuitable, since after several attempts no product was obtained.

Meanwhile glycosylations in the synthesis of another minimal structure analogue which we named furanophostin (discussed in chapter **3**) involving the phosphite donor **47** indicated that a solvent mixture of dioxane/toluene gave improved solubility of the reactants and hence improved yields; accordingly this solvent mixture was used for all the subsequent glycosylations discussed below.

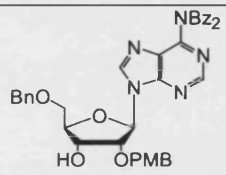
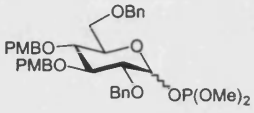
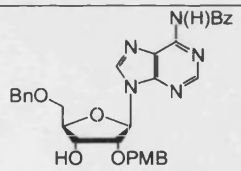
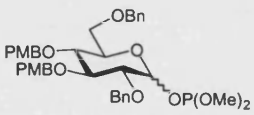
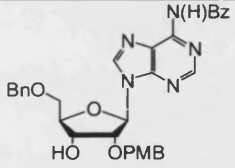
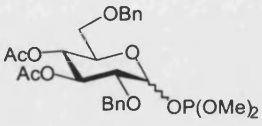
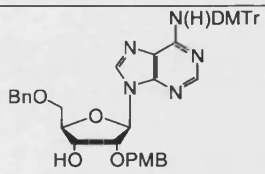
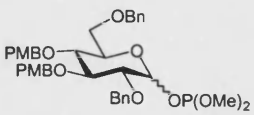
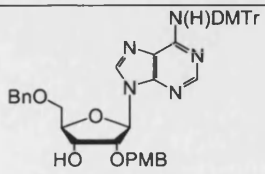
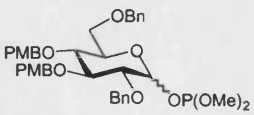
	Acceptor	Donor	Promoter Equ. $AgClO_4/ZnCl_2$	Product number	Yield (%)
1			1.2/0.6	**48**	16
2			1.2/0.6	**49**	29
3			2.4/1.2	**50**	29
4			1.2/0.6	**51**	28
5			2.4/1.2	**52**	53

Table **2.1**: Successful glycosylations.

2.4.1 Initial attempts

Those glycosylations which furnished the desired product are entered in the table above. The first adenosine based acceptor to be investigated was N^6,N^6-dibenzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine. Although the dibenzoyl derivative had been prepared to improve the acceptor solubility, it was found that this molecule was prone to loss of one of the benzoyl groups when exposed to the $AgClO_4/ZnCl_2$ promoter. This lead to a low isolated yield of glycosidation product and intolerance of higher equivalents of $AgClO_4/ZnCl_2$ (see entry 1 in Table **2.1**).

Two other promoters reported for the activation of glycosyl phosphites resulting in α-stereoselective glycosylation were $Ba(ClO_4)_2$,[154] and TMSOTf.[152] Unfortunately glycosylations attempted with either of these reagents failed to give any product.

Since N^6,N^6-dibenzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine was not an ideal acceptor, the mono-benzoyl derivative N^6-benzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine was prepared. Glycosylation of this acceptor in the same conditions as those leading to **48** resulted in a reasonable, although still not acceptable, increase in yield (see entry 2 in Table **2.1**).

The introduction of acyl groups is known to attenuate the reactivity of a glycosyl donor, and it was thought that a less reactive donor would be more compatible with the acceptor. In addition it was thought that an increase in promoter equivalents would compensate for any zinc chloride complexing with the acetate carbonyls. Therefore, a 3,4-di-*O*-actetate phosphite glycosyl donor utilised in chapter **5** for the synthesis of a versatile disaccharide intermediate was employed. But again a disappointing yield was obtained with this combination of donor, acceptor and an increase in the promoter equivalents (see entry 3 in Table **2.1**). However this coupled intermediate would be a reasonable starting point for the introduction of a 3",4"-bisphosphorothioate into an adenophostin A analogue since the 3",4"-di-*O*-acetate is orthogonal to the 2'-*O*-*p*-methoxybenzyl group (see chapter one for the effects of introducing phosphorothioates into Ins(1,4,5)P_3 analogues).

2.4.2 Further attempts

At this time the *p*-methoxybenzylidene acetal was successfully cleaved to give 5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine as discussed previously. The route to this adenosine acceptor was shorter and higher yielding than for the above acceptors, a fact which increased its suitability. The first glycosylation involving this acceptor gave a similar yield to those previously described (see entry 4 in Table **2.1**), but increasing the promoter equivalents to 2.4/1.2 $AgClO_4/ZnCl_2$ led to a much improved yield of 53% (see entry 5 in Table **2.1**).

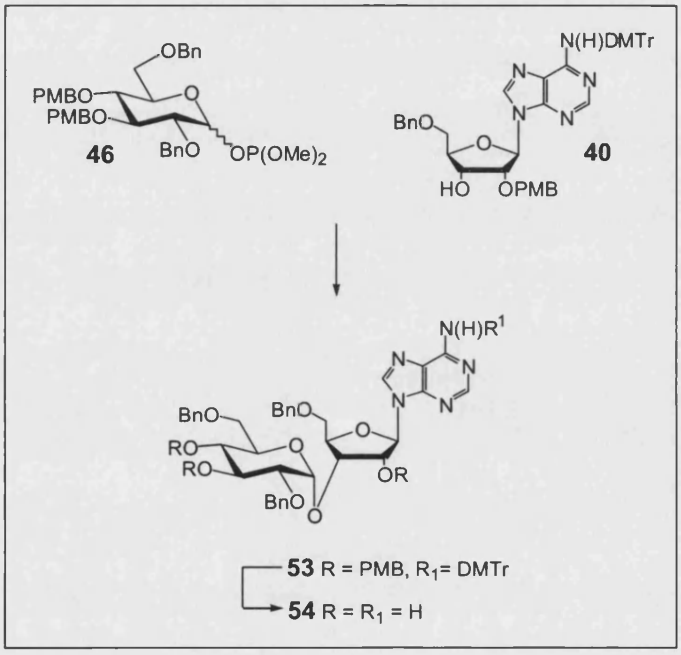


Figure **2.14**: Glycosidation and deprotection

Compound **40** proved to be an extremely important intermediate, as it was also used to prepare derivatives of adenophostin A in which the glucose moiety was replaced by mannose and xylose equivalents, as discussed in later chapters. Thus these glycosylation conditions were used for the synthesis of the target compound adenophostin A. Of note is the complete α-selectivity in all these reactions, and the resulting downfield H-1" product resonances with small axial-equatorial coupling constants, e.g. δ_H 5.22 *J* 3.4 Hz of **53**.

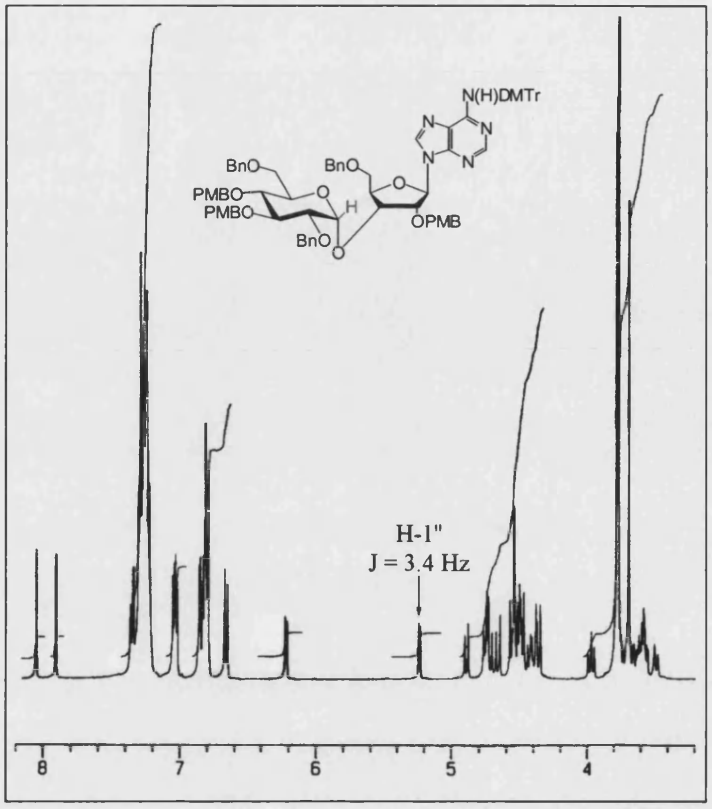


Figure **2.15**: ^{1}H NMR spectrum of **53** indicating the α-anomeric configuration.

Deprotection of the *p*-methoxybenzyl and dimethoxytrityl groups with 10% TFA in DCM, as described for the corresponding deprotection in the synthesis of furanophostin in chapter **3**, led smoothly to the triol necessary for phosphorylation.

2.5 Phosphorylation and Deprotection

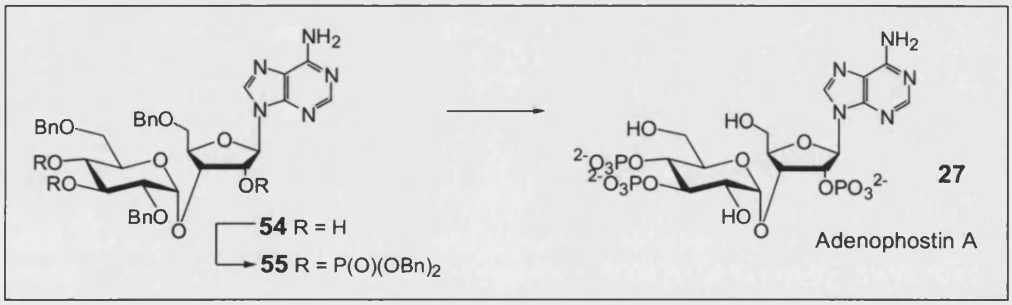


Figure **2.16**: Selective phosphorylation and deprotection of **54** to give adenophostin A

The triol **54** was not protected at the N^6 position, and even though this was achievable with transient protection and benzoylation, with subsequent deprotection it would have imposed two extra steps on the synthesis. Therefore a method for selective phosphorylation of the free hydroxyl groups was sought. In 1991[155] and 1992[156] Letsinger reported the synthesis of oligodeoxynucleotides *via* base-unprotected phosphoramidite monomers where a mixture of pyridinium hydrochloride and aniline or imidazole was used as activator. Although not ideal this method would have been attempted were it not for a very recent publication detailing a more general method for the synthesis of oligodeoxynucleotides without nucleoside base protection.

Hayakawa and Kataoka[157] reported that where a stoichiometric amount of imidazolium triflate was employed as phosphoramidite activator selective hydroxyl phosphitylation was achieved without base protection. It would seem that the reduction in reactivity from the routinely used tetrazole-phosphoramidite complex to the imidazolium-phosphoramidite complex is enough to differentiate between the nucleophilicity of the free ribosyl hydroxyls of **54** and the N^6-position of the adenine base.

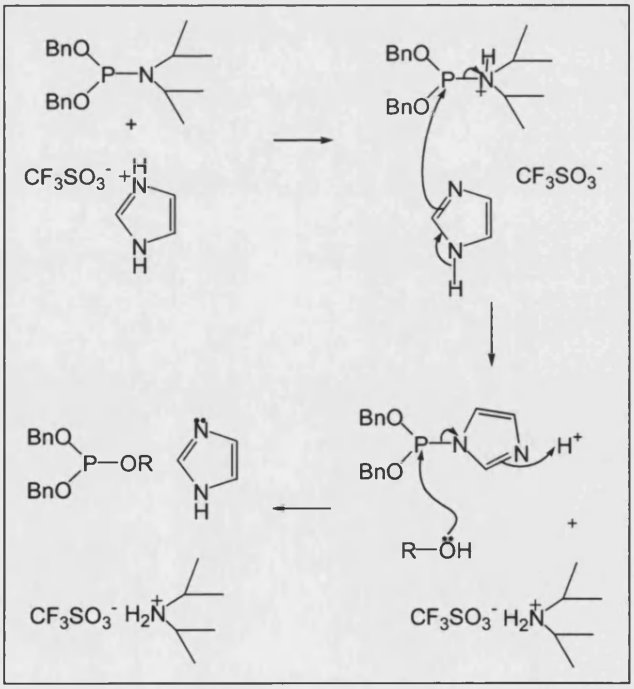


Figure **2.17**: Proposed mechanism for phosphitylation involving imidazolium triflate

Initial protonation of the triflate salt of imidazole was necessary because free imidazole, unlike tetrazole, is not a strong enough acid to protonate the phosphoramidite

reagent and form the diisopropylamine leaving group. However the resulting free imidazole conjugate base is then nucleophilic enough to displace the dialkylamine from the phosphorous atom to give the imidazolide intermediate. Selective phosphitylation ensues with loss of the imidazole moiety as shown in the proposed mechanism in Figure **2.17**.

So this approach was employed in an attempt to phosphitylate the adenophostin A intermediate **54** without base-protection. Imidazolium triflate was easily prepared by slowly adding trifluoromethanesulfonic acid to an equimolar amount of imidazole in DCM. After dilution with diethyl ether the resulting white salt was collected by filtration. Selective phosphitylation was then accomplished with a mixture of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate in DCM. As may be expected, monitoring the reaction by TLC indicated that phosphitylation with imidazolium triflate instead of tetrazole was more sluggish. On complete conversion of starting material into the trisphosphite the reaction mixture was cooled to –78°C before being oxidised with MCPBA. The reaction was quenched at this temperature to avoid any possible oxidation of the adenine base. ^{31}P NMR spectrum of the purified product (**55**) confirmed the presence of three phosphate groups, with the ^{1}H-coupled spectrum exhibiting three sextets. In addition the presence of the free unphosphorylated N^6-position NH_2 was substantiated by the presence of a broad singlet in the ^{1}H NMR at δ_H 6.09 (Figure **2.18**).

Deprotection of the fully protected adenophostin precursor **55** proved difficult, with the benzyl ethers proving particularly stable. Initial attempts with sodium and liquid ammonia were unsuccessful. Catalytic hydrogenation was also less than satisfactory. **55** Was treated separately with 10% palladium on carbon, palladium black and 20% palladium hydroxide, and while the former two catalysts were unsuccessful, the latter was very slow, with complete deprotection taking five days, and this was accompanied by a low yield.

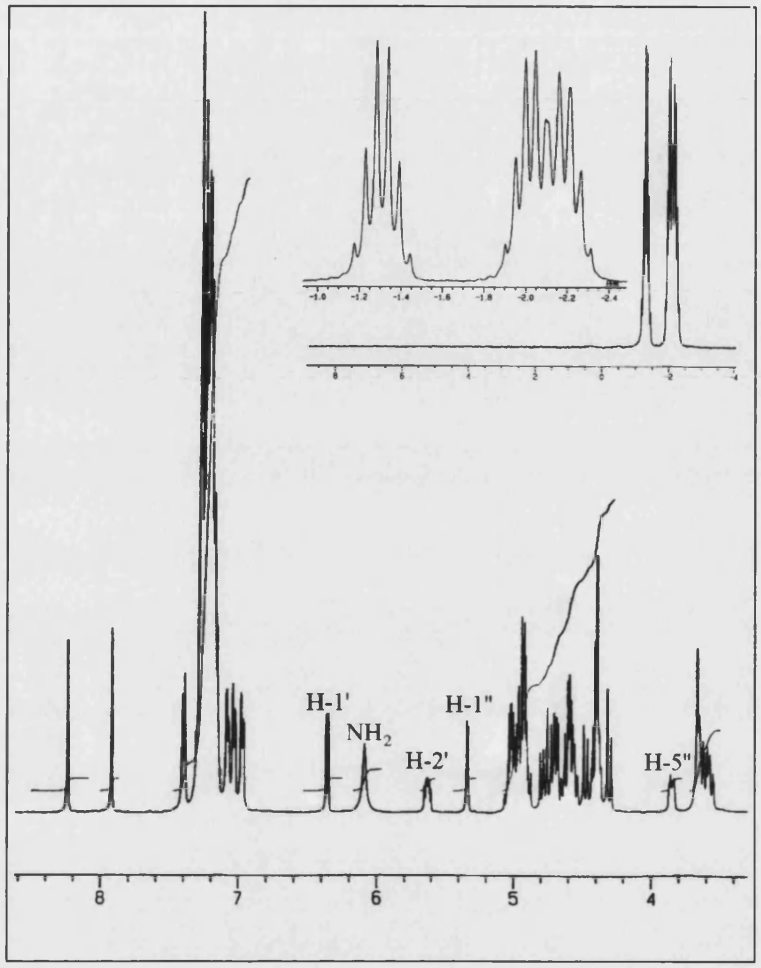


Figure **2.18**: Part of the ^{1}H NMR spectrum of **55** clearly indicating the free N^{6}-amine of adenine and part of the ^{31}P NMR (insert) indicating three protected phosphate groups.

Complete deprotection in high yield was finally achieved with catalytic transfer hydrogenation.[158] A solution of **55** was refluxed in a mixture of methanol, water and cyclohexene with 20% palladium hydroxide for 2hours 30minutes. Following removal of the catalyst the crude product was purified on an MP1 AG ion exchange resin column, being eluted with a gradient of 0-100% 150mM TFA. Adenophostin A was isolated as the free acid and subsequently converted to the sodium salt. Quantification was accomplished by UV assay. ^{31}P NMR spectrum confirmed the presence of three deprotected phosphate groups.

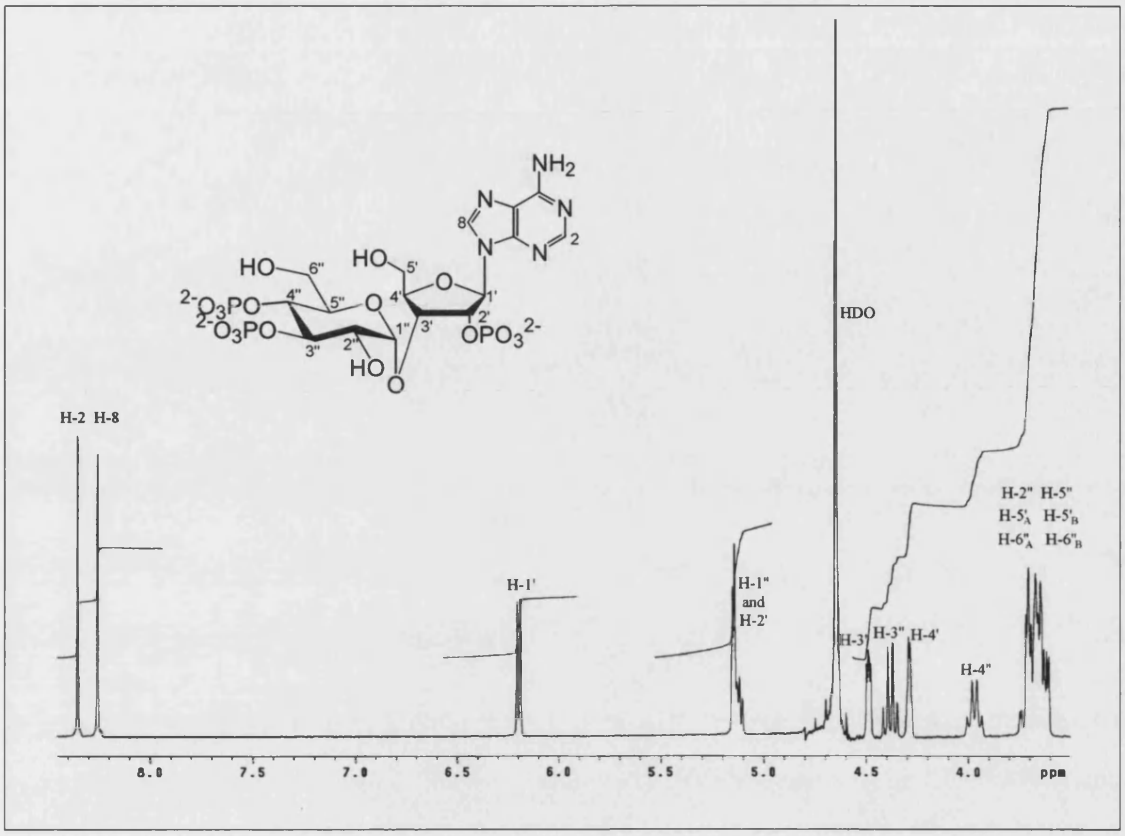


Figure **2.19**: 400MHz ^{1}H NMR spectrum of the free acid of adenophostin A in D_2O.

The ^{1}H NMR spectrum of adenophostin A shown in Figure **2.19** is in keeping with the published data.[159;160] Although it is worth noting that the signals of some of the protons are not exactly the same as those found in the literature. This is probably due to differences in pH between this sample solution and those prepared by other groups, since the ^{1}H NMR spectrum of adenophostin A shown in Figure **2.19** is of the free acid, and the published NMR data is of sodium salts.

This synthetic adenophostin A was compared with an authentic sample of natural adenophostin A by reverse-phase HPLC using an ODS column. The eluent was a gradient of acetonitrile and phosphate buffer, containing 0.1% w/v of tetrabutylammoniumhydrogen sulphate, acting as an ion-pair reagent. The starting concentration was 10% acetonitrile/90% phosphate buffer and the gradient was run over 20 minutes, finishing at 40% acetonitrile/60% phosphate buffer, with any eluting material being detected by a UV spectrometer set at 259 nm. The resulting traces are shown in Figure **2.20**.

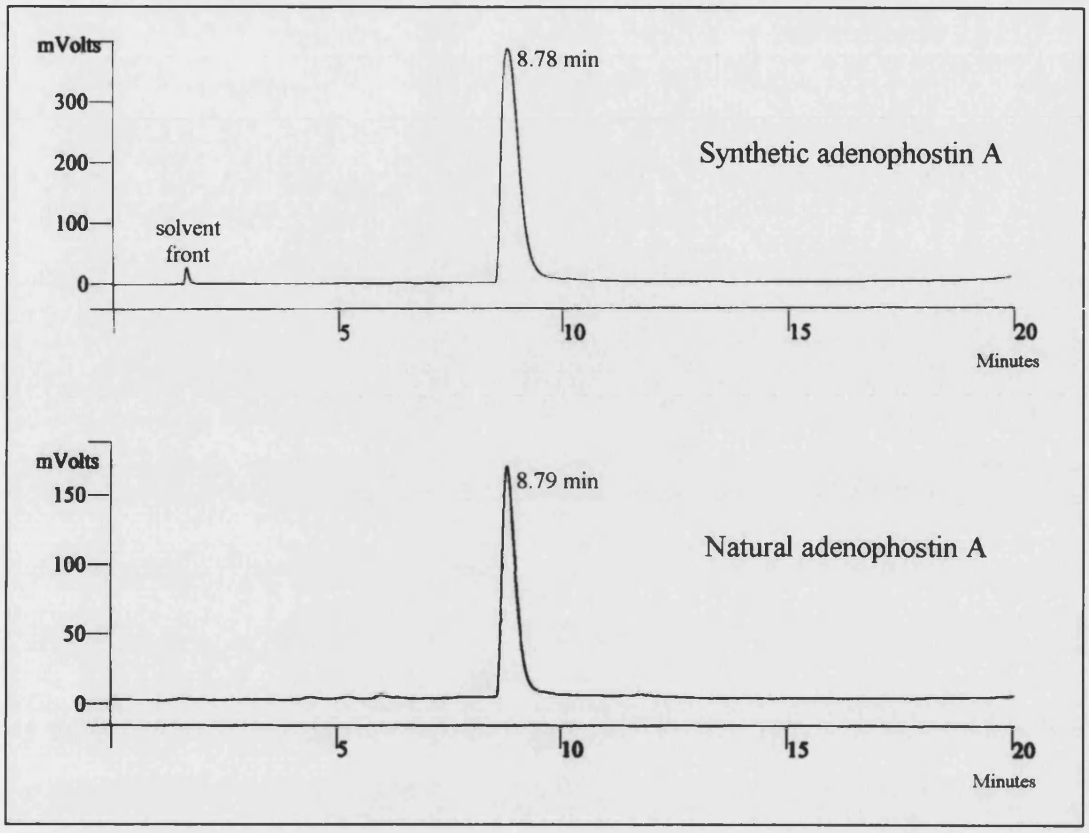


Figure **2.20**: Comparison of synthetic and natural adenophostin A by HPLC.

It is clear from the preceding figure that the synthetic adenophostin A has a similar retention time to that of the natural material. The sample of synthetic adenophostin A was shown to be 98% pure while the natural adenophostin A was 96% pure.

2.6 Biological results

The ability of a sample of the synthetic adenophostin A to release Ca^{2+} from permeabilised hepatocytes was compared with that of a sample of natural adenophostin A and the results are shown in Table **2.2**. The method used by our collaborators was as follows: Permeabilised hepatocytes were loaded to steady state (5min at 37°C) with $^{45}Ca^{2+}$ in a cytosol-like medium (CLM: KCl, 140mM, NaCl, 20mM, 2mM $MgCl_2$, 1mM EGTA, 300μM $CaCl_2$, 20mM Pipes, pH7.0) containing ATP (1.5mM), creatine phosphate (5mM) creatine phosphokinase (5units/ml) and FCCP (10μM). After 5min, thapsigargin (1.25μM) was added to the cells to inhibit further Ca^{2+} uptake, 30s later the cells were added to appropriate concentrations of the agonists and after a further 60s the

$^{45}Ca^{2+}$ contents of the stores were determined by rapid filtration. Concentration-response relationships were fitted to a four parameter logistic equation using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}) and Hill slope (h) were determined. All results are expressed as means±SEM.

	EC_{50} (nM)	h	% release	n
Natural adenophostin A	9.2±1.7	2.99±0.32	49±3	3
Synthetic adenophostin A	9.6±1.0	2.46±0.19	50±1	3

Table **2.2**: Ca^{2+} release from permeabilised hepatocytes

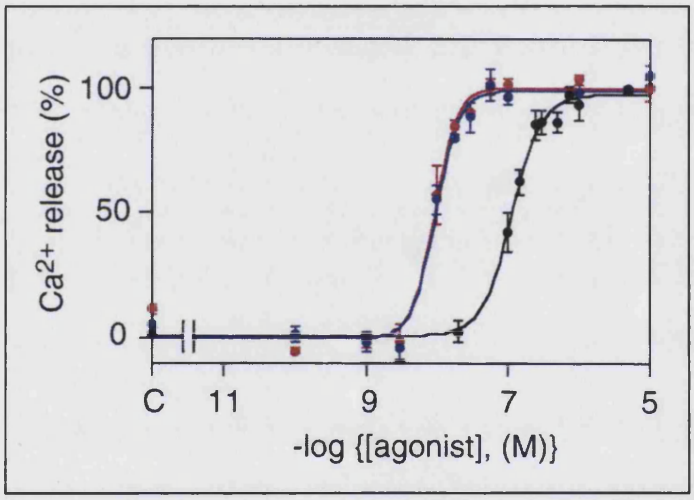


Figure 2.21: Ca^{2+} mobilization evoked by natural and synthetic adenophostin A, and Ins(1,4,5)P_3.

The results (means ±sem, n = 3 or 4) show the concentration-dependent effects of Ins(1,4,5)P_3 (black), natural (blue) or synthetic (red) adenophostin A on Ca^{2+} release from the Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} stores of permeabilized rat hepatocytes.

It was clear from the Ca^{2+} releasing activities of natural and synthetic adenophostin A presented in Table **2.2** and Figure **2.21** that our synthetic adenophostin A was equipotent with natural adenophostin A.

In an ongoing collaboration with Dr I. Bezprozvanny, University of Texas, it has been shown that this synthetic adenophostin A can interact with reconstituted cerebellar

$Ins(1,4,5)P_3$ receptors in lipid bilayers in an unusual way, in conjunction with the observation of prolonged open-states for some receptors.

2.7 Potentiometric and NMR studies of adenophostin A

The ionisation of $Ins(1,4,5)P_3$ at the $Ins(1,4,5)P_3$ receptor is thought to be particularly influential in both its conformation and its interactions with specific residues of the binding pocket. Several NMR studies on *myo*-inositol phosphates have been reported by our group in collaboration with B. Spiess[161-163] in which the conformation of an inositol phosphate in relation to its degree of protonation as a function of pH has been studied.

It was therefore thought that similar examination of adenophostin A would reveal whether or not the ionisation of adenophostin A at physiological pH was different to that of $Ins(1,4,5)P_3$. Any differences in ionisation may potentially lead to a possible explanation for the exceptional activity of adenophostin A. Therefore, a sample of our synthetic adenophostin A was examined by B. Spiess using potentiometric and NMR titrations.

Adenophostin A carries, in addition to the protonatable nitrogen, three phosphate groups, each group being able to bind one proton for pH values ranging from 12 to 2.5. Thus, in the latter pH range, four macroscopic overall protonation constants quantify the protonation process. These constants cannot be attributed to a given protonation site, since most of them are less than two log units apart and therefore a given macroscopic protonation step involves two or even three different basic sites. An inframolecular approach, which aims at defining the intrinsic acid-base properties of each group requires the resolution of a more detailed protonation scheme as shown in Figure **2.22**. In that figure, the first protonation steps, which refer to the three phosphate groups, have to be described by eight microspecies and twelve related micro-protonation constants, with the fourth equivalent of protons mainly binding to an adenine nitrogen.

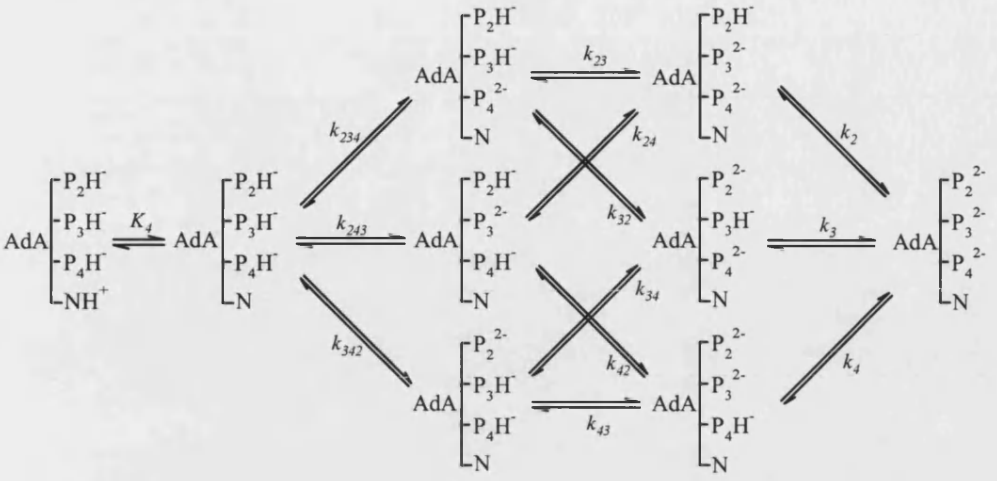


Figure **2.22**: Protonation scheme for adenophostin A.

^{31}P NMR can be used to study individual protonation, provided that the observed chemical shifts for the phosphorus resonances mainly depend on the electronic effects accompanying the variations in the protonation states. B. Spiess, using ^{31}P NMR and potentiometric titrations, calculated the protonated fraction of the phosphate groups of adenophostin A in relation to pH and these are compared with the protonated fractions of Ins(1,4,5)P_3 in Figure **2.22**, where $f_{i,p}$ is the protonated fraction of a phosphate group in position i on adenophostin A and Ins(1,4,5)P_3.

Consideration of the fraction of the protonated phosphates of adenophostin A at positions 2', 3" and 4" at nearly physiological pH (pH = 7.5) gave 5%, 61%, and 26% respectively. Remarkably, the protonated fractions for the equivalent phosphates of Ins(1,4,5)P_3 were 8%, 57%, and 26% (vertical line in Figure **2.23**). It was really surprising to observe such close values, knowing that minor structural variations in inositol phosphates can largely affect these percentages.

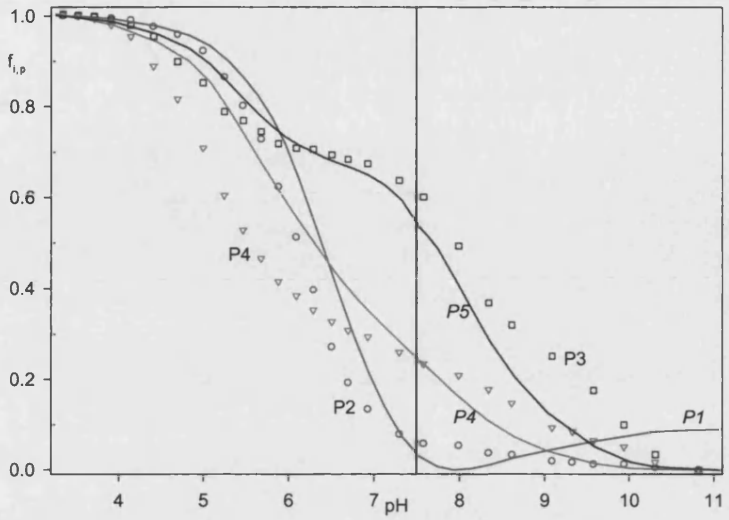


Figure **2.23**: Protonation fraction curves vs pH of adenophostin and Ins(1,4,5)P_3. The curves in solid line correspond to the protonation fraction curves of Ins(1,4,5)P_3 (*P1*, *P4*, *P5*). The curves in circles, squares and triangles correspond to the protonation fraction curves of adenophostin A (*P2*, *P3*, *P4*).

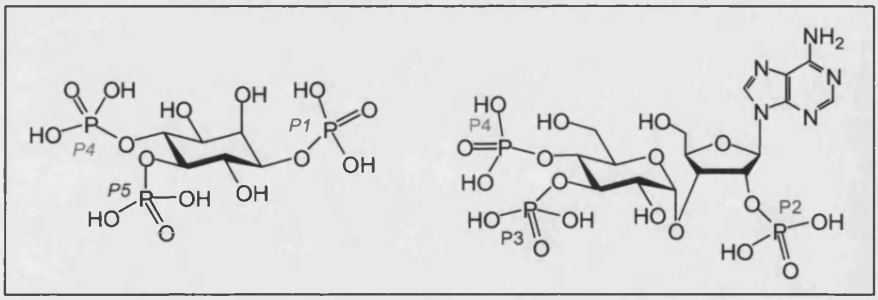


Figure **2.24**: The structures of Ins(1,4,5)P_3 and adenophostin A.

We can imagine that the prime interactions of Ins(1,4,5)P_3 with its binding pocket in the Ins(1,4,5)P_3 receptor are made *via* the charged phosphate groups. These results have shown that the ionisation of the phosphate groups of Ins(1,4,5)P_3 and adenophostin A is similar at nearly physiological pH, and therefore that their ionic interactions at the Ins(1,4,5)P_3 receptor are presumably also similar. Thus, it may be concluded that the exceptional activity of adenophostin A cannot be explained by a difference in the ionisation state of its phosphate groups in comparison with those of Ins(1,4,5)P_3.

Chapter three

Minimal structure analogues

3 Minimal structure analogues

3.1 Introduction

In between the discovery of adenophostin A and the beginning of this work a few analogues of adenophostin A were reported (see Figure **3.1**). All of these analogues lacked the adenine base or a similar structure and were therefore termed minimal structure analogues. Moreover all were based on carbohydrates with the exception of 6-deoxy-6-hydroxymethyl-*scyllo*-inositol 1,2,4-trisphosphate[164](**56**) which was based on *scyllo*-inositol. **56** May be considered as an inositol based analogue of the adenophostin A glucose motif. If the glucopyranoside ring of adenophostin A is considered as analogous to the Ins(1,4,5)P_3 cyclohexane ring then the adenophostin A 3",4" bisphosphate becomes equivalent to the 4,5 bisphosphate of Ins(1,4,5)P_3. Therefore the position of adenophostin A most likely to correspond to the position 3-hydroxyl of Ins(1,4,5)P_3 carries a sterically bulky hydroxymethyl group. **56** Was designed to explore the effect of introducing such a group into an Ins(1,4,5)P_3-like structure. One report concludes that there is little tolerance at the Ins(1,4,5)P_3 receptor for an increase in steric bulk at position 3 of Ins(1,4,5)P_3. Investigation into alkylation at this position with a series of racemic 3-*O*-alkylated analogues[165] found that increasing steric bulk of the equatorial 3-position produced analogues with progressively decreasing activity at the Ins(1,4,5)P_3 receptor. However in contrast to this, D-3-*C*-trifluoro-methyl-*myo*-inositol 1,4,5-trisphosphate, which has an added axial 3-CF_3-group, was almost equipotent to Ins(1,4,5)P_3[165] suggesting that there may be tolerance in the Ins(1,4,5)P_3 receptor binding pocket for bulky axial substituents at the 3 position. Furthermore the Ins(1,4,5)P_3 analogue D-2,3-methoxymethylene-*myo*-inositol 1,4,5-trisphosphate has also been shown to be almost as active as Ins(1,4,5)P_3 at the Ins(1,4,5)P_3 receptor.[166]

Biological evaluation of racemic **56** in permeabilised rabbit platelets found that it exhibited equal potency in Ca^{2+} release to Ins(1,4,5)P_3, and in binding assays in rat cerebellar membranes **56** was also found to be equipotent to Ins(1,4,5)P_3. These findings demonstrate that modification of the 3-position of an analogue similar to Ins(1,4,5)P_3 with a hydrophilic hydroxymethyl group is well tolerated by the Ins(1,4,5)P_3 receptor and this is in keeping with its similarity to the glucose 5"-structure in adenophostin A. It is important to note here that **56** bears one other structural difference to Ins(1,4,5)P_3, in

that the 2-position is equatorial as opposed to axial. Although it has been demonstrated that the 2 position of Ins(1,4,5)P_3 is relatively unimportant to the activity of Ins(1,4,5)P_3.[167] With this in mind it is better to compare the activity of **56** with *scyllo*-inositol 1,2,4 trisphosphate. Racemic **56** exhibits about four times greater potency and 10-fold higher binding affinity than *scyllo*-Ins(1,2,4)P_3 at the Ins(1,4,5)P_3 receptor.[168] This finding means that a hydroxymethyl group at the position corresponding to position 3 of Ins(1,4,5)P_3 slightly increases Ca^{2+}-mobilising potency and markedly increases affinity for the Ins(1,4,5)P_3 receptor. It is possible that the equatorial 2-hydroxyl of **56** allows accommodation of certain larger substituents at the 3-position, and in adenophostin A the analogous position is the relatively small glucopyranoside ring oxygen, so indeed the role of the 2- and 3-position substituents may be closely connected. In addition the acetate ester of the hydroxymethyl present in adenophostin B, seems to have no effect on activity at the Ins(1,4,5)P_3 receptor.

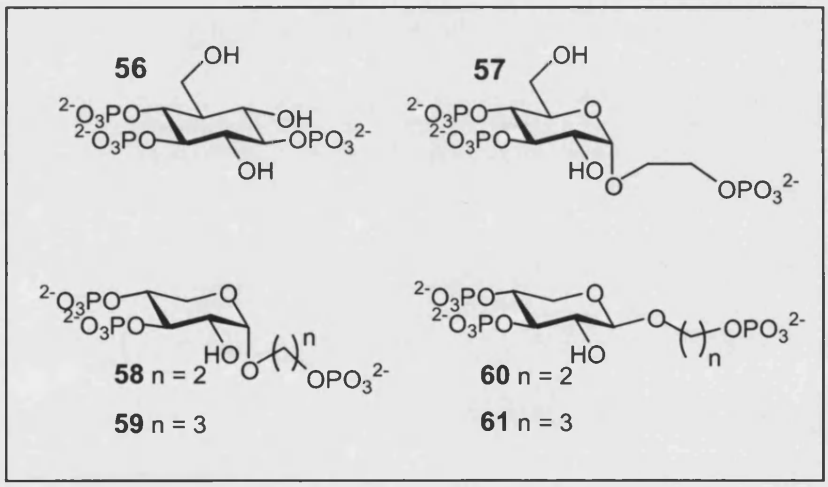


Figure **3.1**: Minimal structure analogues of adenophostin A

The first carbohydrate based adenophostin A analogue, 2-hydroxyethyl α-D-glucopyranoside 2',3,4-trisphosphate [Gluc(2'3,4)P_3, **57**],[168-171] in which the adenosine structure had been effectively removed, had an hydroxyethyl phosphate at the glucose anomeric position to mimic the 2' phosphate on the ribose ring of adenophostin A. Initial testing in rabbit platelets found Gluc(2'3,4)P_3 to be a full agonist at the Ins(1,4,5)P_3 receptor with a potency *ca.* 10-fold lower than that of Ins(1,4,5)P_3. These results were later confirmed by another group who carried out a more extensive biological evaluation of Gluc(2'3,4)P_3 in SHSY5Y neuroblastoma cells.[172;173] Molecular modeling studies on

Gluc(2'3,4)P_3 confirmed that the conformationally flexible hydroxyethyl phosphate was likely to adopt an extended conformation, therefore preventing the 2'-phosphate from accurately mimicking either the 1-phosphate of Ins(1,4,5)P_3 or the 2'-phosphate of adenophostin A. Thus Gluc(2'3,4)P_3 is a 100-fold weaker agonist at the Ins(1,4,5)P_3 receptor than adenophostin A. Although it may be possible that this is due just to the mobility of the ethylphosphate motif, it is more probable that the relatively low potency stems from a combination of a lack of the rigidity that is provided by the ribofuranoside ring and the lack of an adenine base structure.

Another study was later published outlining the synthesis and biological activity of a group of xylose analogues closely related to Gluc(2'3,4)P_3.[174] The xylose equivalent of Gluc(2'3,4)P_3 (**58**), in which the 5-hydroxymethyl was absent, exhibited an EC_{50} value in permeabilised hepatocytes of 0.43μM, about 10-fold weaker than Ins(1,4,5)P_3. Other similar analogues in this series (**59–61**) where the aglycon was increased in length or the anomeric configuration inverted to β were all weaker than Gluc(2'3,4)P_3 and its xylose equivalent. Unfortunately it is difficult to draw firm conclusions by comparison of these results as the tests were carried out in different cell models, although it is tempting to suggest that the 5"-hydroxymethyl of adenophostin may not be so crucial to its activity. This is supported by the finding that 3-deoxy-Ins(1,4,5)P_3 is a relatively potent Ins(1,4,5)P_3 agonist. Further consideration of this point is in chapter **4** where the biological results of 'xylophostin' are discussed.

Following on from these initial syntheses it was decided that more rigid structures should be prepared lacking the adenine base.

3.2 Synthesis of Ribophostin

3.2.1 Introduction

The most intriguing feature of adenophostin A's structure is the adenine base, as nothing in the structure of Ins(1,4,5)P_3 resembles it. This suggests that it plays an important role in the exceptional biological activity of adenophostin A, possibly by engaging with a nearby region of the Ins(1,4,5)P_3 receptor and stabilising binding interactions, and/or optimising the position of the 2'-phosphate at the binding site.

Therefore, an analogue in which the adenine has been effectively deleted, leaving the ribose ring intact was an attractive target. We named this analogue "ribophostin" (**62**).

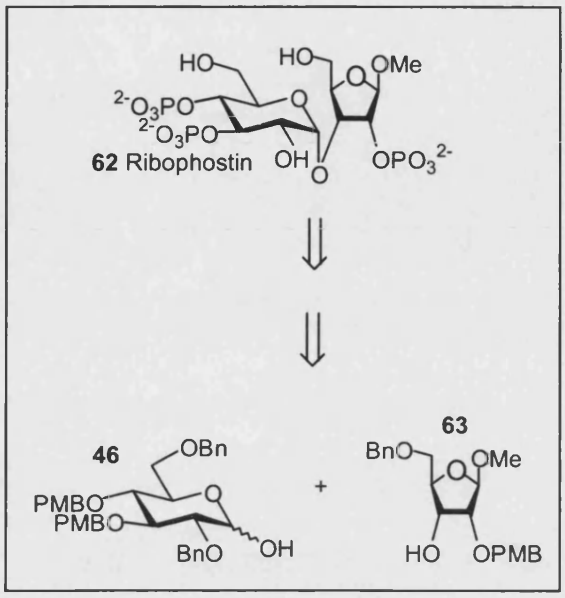


Figure **3.2**: Retrosynthetic basis for the synthesis of ribophostin indicating the required glycosyl donor precursor and acceptor.

Disconnection of the adenophostin A structure with complete loss of the adenine base would be the most logical target, leaving a derivatised tetrahydrofuran ring as the required acceptor. Although plausible, it was decided that a synthetically simpler approach would be adopted where the adenine was replaced with OMe. The disaccharide **62** constructed from a D-glucopyranosyl donor and methyl-β-D-ribofuranoside acceptor was therefore designated as a suitable target. Work previously carried out be D. J. Jenkins provided the ideal route to the protected glucopyranose intermediate, and the acceptor was already in hand in our laboratory.

3.2.2 Synthesis of the donor

For the preparation of this adenophostin A analogue, imidate glycosylation methodology was adopted. Work carried out prior to this project by D. J. Jenkins[170] outlined a route to a selectively protected glucose intermediate amenable to conversion into the desired glucosyl donor. Thus 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose (**46**) was prepared on a large scale following the published procedures.[170] Briefly, Fischer glycosidation of D-glucose with allyl alcohol in the presence of a strong

cation-exchange resin yielded a mixture of allyl α-and β-D-glucopyranoside products (**64**) in a ratio of 7:3 as estimated from the integral ratio of the anomeric protons in the ^{1}H NMR spectrum. Direct benzylation of this mixture was then carried out by way of a bis-stannylene intermediate followed by stirring with neat benzyl bromide for three days at 80–90°C. After removal of benzyl bromide by column chromatography the desired allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**65**) was isolated by crystallisation from diisopropyl ether in 10% yield with melting point of 73–75°C (lit.[170] 74–77°C). Although the yield was rather low, as expected from the literature, this is a convenient method to produce this intermediate on a large scale.

Conversion into allyl 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (**67**) with sodium hydride and *p*-methoxybenzyl bromide was easily achieved. This was followed by removal of the allyl aglycone by a two step method.[175] First the allyl group was isomerised to the *cis*-prop-1-enyl glucopyranosides, and then this acid labile group was cleaved using acid hydrolysis to furnish the fully protected glucose intermediate (**46**) (melting point 117–120°C, lit.[170] 120–131°C) ready for conversion into the glucosyl donor.

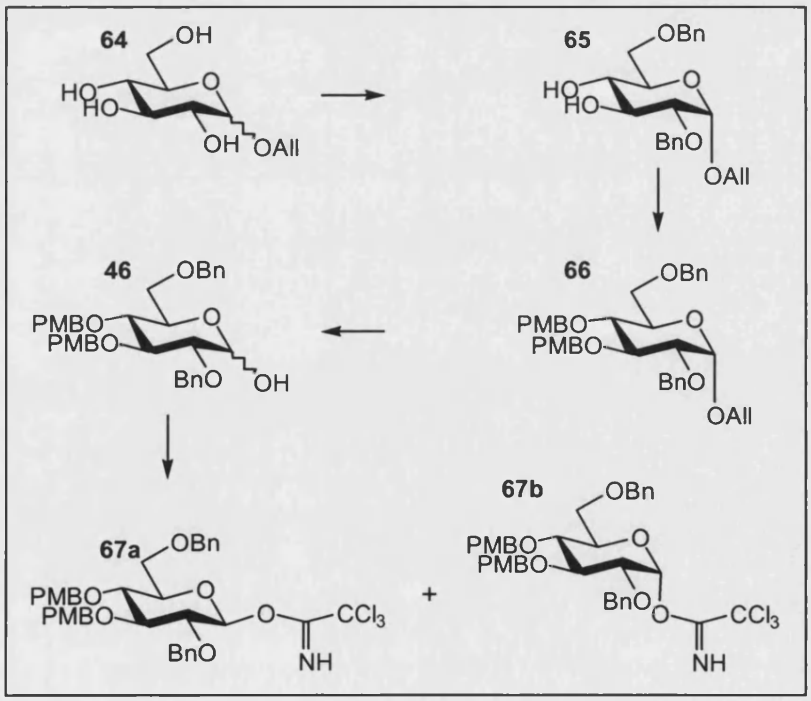


Figure **3.3**: Route to trichloroacetimidate **67a**.

46 Was then converted into its β-trichloroacetimidate. Of the two possible anomers this is the most difficult to prepare. Under reaction conditions reported by R. R. Schmidt and J. Michel[176] for tetra-*O*-benzyl-D-glucopyranose the kinetic product (β-anomer) is formed first. Thus the presence of a relatively mild base, in this case potassium bicarbonate, results in the formation of the β-anomer, but this is accompanied by slow conversion into the α-anomer, which is the thermodynamic product. Therefore the reaction was monitored closely by TLC and quenched when the optimum conversion to the β-anomer was observed, before the formation of too much of the α-anomer. Flash chromatography yielded the desired 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl trichloroacetimidate (**67**) in only moderate yield (48%), accompanied by a notable amount of the α-anomer. Configurations of the two anomers were easily assigned on the basis of their ^{1}H NMR spectra. The axial H-1 of the β-anomer exhibited a typically large coupling constant *J* 8.3 Hz and was upfield (δ_H 5.78) of the equatorial H-1 of the α-anomer, δ_H 6.51, *J* 3.3 Hz. The trichloroacetimidate NH proton was also clear in the spectra for both anomers.

After repeated attempts at the preparation of this glycosyl donor the yield could not be improved and since plenty of the starting glucopyranose had been using the large scale procedure described above, the yield of glycosyl donor was accepted and the synthesis continued.

3.2.3 Synthesis of the acceptor

A route to an appropriate acceptor, methyl 2-*O*-allyl-5-*O*-benzyl β-D–ribofuranoside has recently appeared in the literature.[171] However a co-worker, D. J. Jenkins, prepared the corresponding 2-*O*-*p*-methoxybenzyl ether (**63**) by a shorter route in five steps from D-ribose as shown in Figure **3.4**. Briefly, D-ribose was converted to the known methyl β-D–ribofuranoside which was reacted with 1.05 equivalents of *p*-methoxybenzaldehyde dimethyl acetal to yield the 2,3-*O*-*p*-methoxybenzylidene derivative. 5-*O*-Benzylation and subsequent reductive cleavage of the *p*-methoxybenzylidene with either $LiAlH_4$-$AlCl_3$ in refluxing THF or DIBAL-H in DCM gave the desired acceptor and its regioisomer in approximately equal proportions.

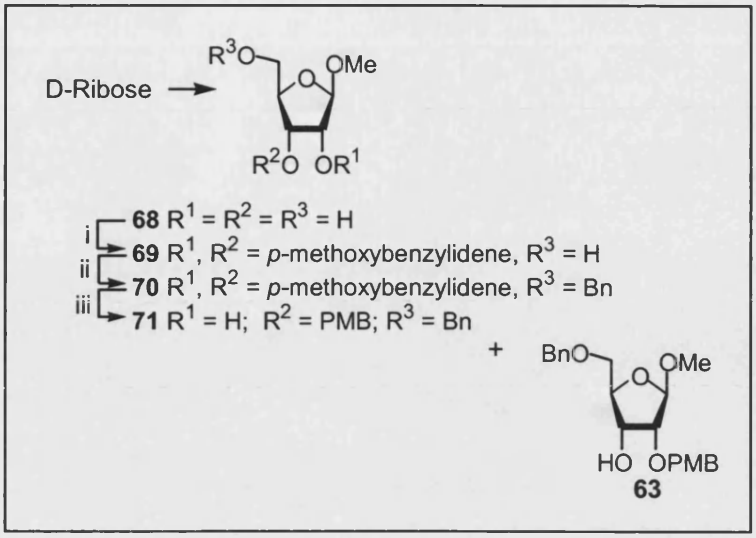


Figure **3.4**: Route to glycosyl acceptor **63**.

Reagents and conditions: i) *p*-methoxybenzylidene dimethyl acetal (1.05 equiv.), PTSA, DMF, 70 °C; ii) NaH, BnBr, DMF; iii) DIBAL-H (2.5 equiv.), CH_2Cl_2, –78 °C.

3.2.4 Glycosidation and deprotection

With suitable imidate glycosyl donor and acceptor in hand the glycosidation was carried out under conditions established as those that generally favour α-stereoselectivity,[177] that is at room temperature in diethyl ether in the presence of catalytic TMSOTf. The reaction proceeded smoothly to give predominantly the α-D-glucopyranosyl isomer (yield of mixture 54%), and the H-1' signal in the ^{1}H NMR spectrum confirmed the formation of the α-coupled product with a typically small axial-equatorial coupling constant of J 3.4 Hz. The β-D-glucopyranosyl isomer constituted *ca.* 20% of the isolated product as judged by ^{1}H NMR integrals and unfortunately, it was not possible to remove this undesired product at this point. It was decided to proceed with the deprotection of the *p*-methoxybenzyl groups to give the desired triol ready for phosphorylation, in the hope that the anomeric mixture of triols could be separated.

In the synthesis of Ins(1,4,5)P_3 analogues *p*-methoxybenzyl groups are often used to mask the hydroxyls designated for phosphorylation towards the end of a synthetic route, because they are easily removed in the presence of other protecting groups such as benzyl ethers. This approach has been adopted here also. Oxidative cleavage of *p*-methoxybenzyl ethers is the most frequent method of deprotection and can be achieved with either DDQ or ceric ammonium nitrate. At this time DDQ was considered the best reagent to furnish the desired triol.

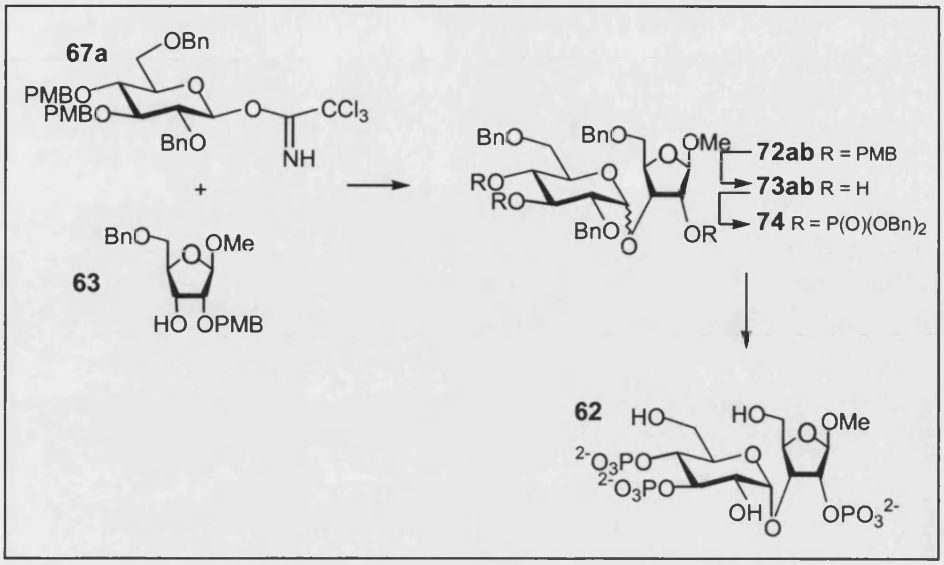


Figure **3.5**: Synthetic route to ribophostin (**62**).

Once treated with DDQ in wet dichloromethane the resulting α-D-glucopyranosyl triol (**73**) could be separated from its β-isomer by flash chromatography leaving the pure triol for phosphorylation.

Since this work was carried out details of a method of *p*-methoxybenzyl cleavage using catalytic amounts of DDQ have appeared in the literature.[178] $FeCl_3$ used for the oxidation of hydroquinones to quinones was used to oxidise the by-product dichloro dicyano hydroquinone (DDHQ) back to DDQ. Reducing the amount of DDQ in a reaction overcomes the two main disadvantages of its use, first the by-product DDHQ is often difficult to get rid of, and second DDQ is a relatively expensive and toxic reagent.

3.2.5 Phosphorylation and Deprotection

The triol **73** was phosphitylated in the usual way with bis(benzyloxy) (diisopropylamino)phosphine and tetrazole to give a trisphosphite intermediate. Oxidation with MCPBA at reduced temperature resulted in the fully protected trisphosphate (**74**) in good yield after purification. ^{31}P NMR confirmed the presence of three phosphate groups with the coupled spectrum exhibiting three sextets.

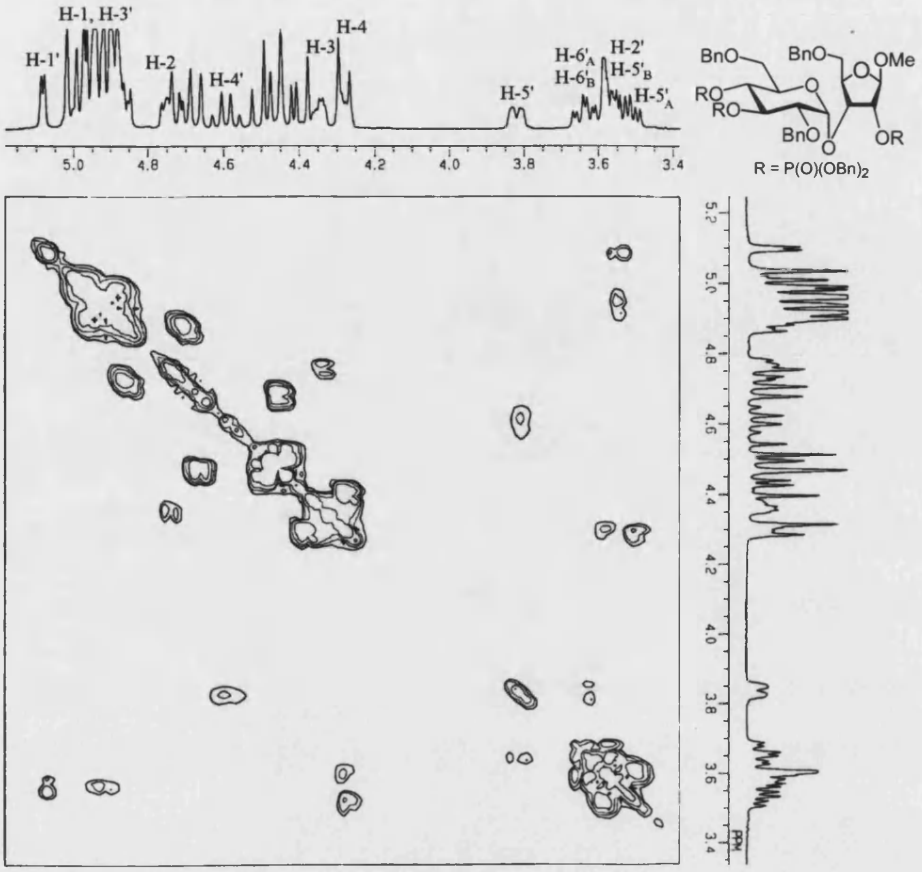


Figure **3.6**: Part of the ^{1}H-^{1}H COSY NMR spectrum of **74** in $CDCl_3$.

The final deprotection was achieved by hydrogenation over a palladium catalyst. Purification of the product by ion exchange chromatography afforded the triethylammonium salt of **62**. Strangely, this salt was not soluble in water (not even partially), and for quantification purposes aqueous solubility was essential. Therefore it was converted into the freely water soluble potassium salt by addition of 0.1M aqueous potassium hydroxide. The volume added was calculated based on the ^{1}H NMR spectrum in which a ratio of triethylammonium ions : disaccharide of 3.5:1 was observed. Thus quantification by the Briggs phosphate assay was made possible.[179]

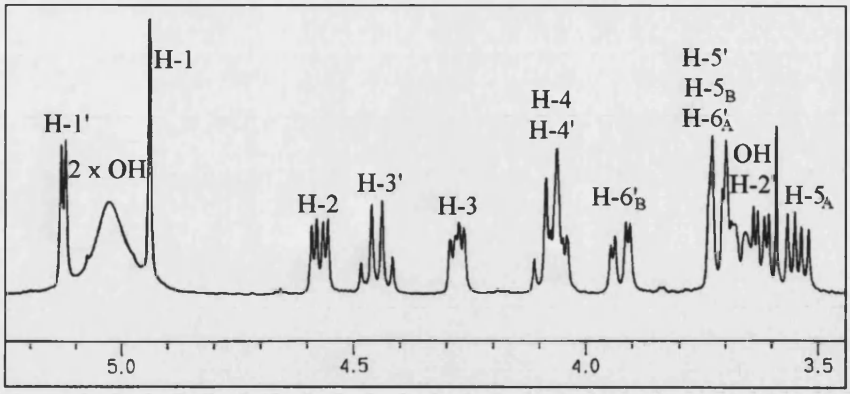


Figure **3.7**: Part of the ^{1}H NMR spectrum of the triethylammonium salt of ribophostin in CD_3OD.

3.3 Biological results

Compound	EC_{50} (nM)	h	n
Ins(1,4,5)P_3	153.13±11.08	2.25±0.20	7
Ribophostin	213.35±7.41	2.49±0.18	3

Table **3.1**: Unidirectional $^{45}Ca^{2+}$ efflux in permeabilised hepatocytes. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[180] Results are shown as means ± S.E.M.

Biological evaluation of ribophostin by a collaborator using the method described for adenophostin A indicated that it was a full agonist at the Ins(1,4,5)P_3 receptor with a potency for releasing intracellular Ca^{2+} from permeabilised hepatocytes almost the same as Ins(1,4,5)P_3 (Table **3.1**). Ribophostin was also shown in equilibrium binding studies with hepatic membranes to completely displace specific [^{3}H]Ins(1,4,5)P_3 binding with equal potency to Ins(1,4,5)P_3 (Table **3.2**). Figure **3.8** depicts the specific [^{3}HIns(1,4,5)P_3] binding to rat hepatic membranes in the presence of the indicated concentrations of adenophostin A (□) or ribophostin (□).

Compound	K_d (nM)	h
Ins(1,4,5)P_3	8.65±0.89	0.96±0.04
Ribophostin	8.42±2.5	0.89±0.10
Adenophostin A	0.87±0.20	1.28±0.37

Table **3.2**: The K_d values were determined from equilibrium competition binding experiments with [^{3}H]Ins(1,4,5)P_3 in the presence of and ribophostin or Ins(1,4,5)P_3 in hepatic membranes. Results are shown as means ± S.E.M.

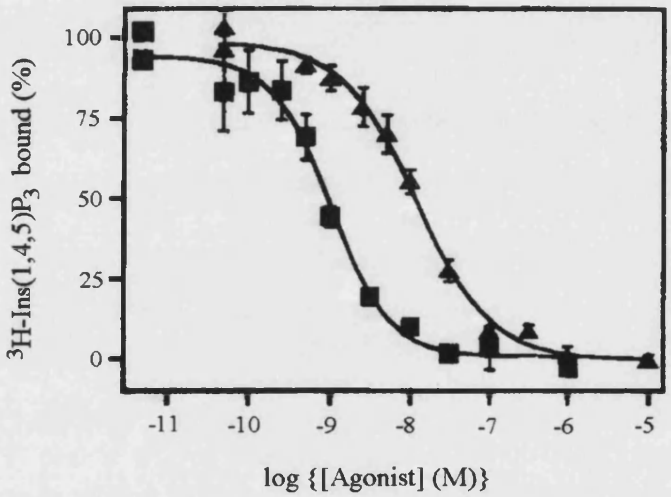


Figure **3.8**: Specific [^{3}HIns(1,4,5)P_3] binding to rat hepatic membranes is shown in the presence of the indicated concentrations of adenophostin A (■) or ribophostin (▲).

3.4 Synthesis of furanophostin

3.4.1 Introduction

It was clear from molecular modelling studies that the rather mobile hydroxyethylphosphate of Gluc(2',3,4)P_3 (**57**) did not achieve good positioning of the 2'-phosphate at the Ins(1,4,5)P_3 receptor binding site. It was therefore decided that a more rigid structure would be prepared to investigate how this might affect binding to the Ins(1,4,5)P_3 receptor. We were also interested in pruning the structure of ribophostin to find out if the 4'-hydroxymethyl group was necessary for its Ins(1,4,5)P_3-like activity, and if the anomeric *O*-methyl group was detrimental to binding in any way. Thus we developed a synthesis of [(3*S*,4*R*)-3-hydroxytetrahydrofuran-4-yl] α-D-glucopyranoside 3,3',4-trisphosphate (furanophostin), an analogue that lacks both the *O*-methyl and 4'-hydroxymethyl moieties of ribophostin, but retains the rigidity of a ribofuranose ring.

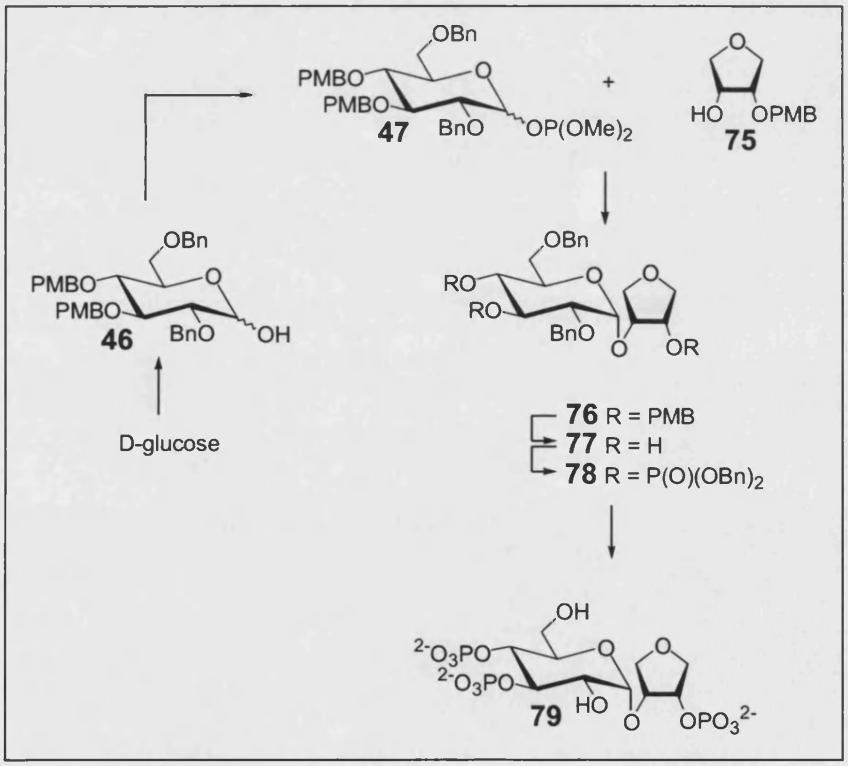


Figure **3.9**: Synthetic route to furanophostin.

3.4.2 Synthesis of the donor

At the time this synthesis was undertaken we thought that phosphite glycosidation methodology would be preferable to the use of trichloroacetimidates, since the glycosyl phosphite donor was much easier to prepare in quantitative yield and the reported α-stereoselectivity for glycosidations with phosphite donors has been shown to be comparable to those using trichloroacetimidate donors[151] (also see chapter **2**). Therefore 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose was converted into its dimethyl phosphite as described in the synthesis of adenophostin A.

3.4.3 Synthesis of the acceptor

This acceptor (**75**) was prepared by A. M. Riley. Although the structure is simple the synthesis required optical resolution of an enantiomeric intermediate, and the conversion of one enantiomer into a compound for which the absolute configuration was already known. The route followed is outlined in Figure **3.10**.

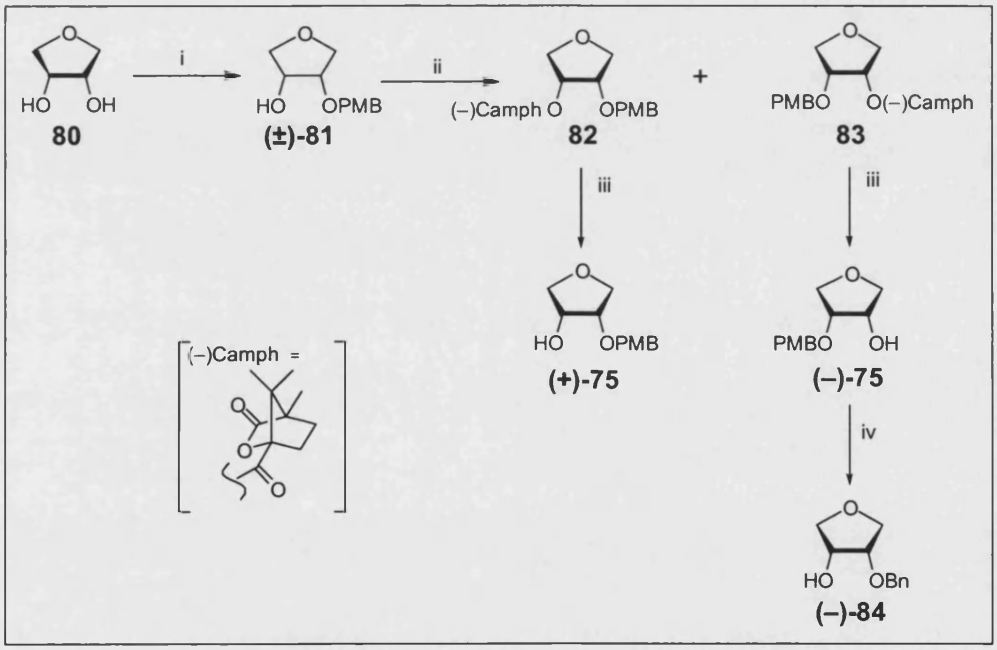


Figure **3.10**: Synthetic route to glycosyl acceptor **(+)-75**.

Reagents and conditions: i) a) *p*-methoxybenzylidene dimethyl acetal (1.05 equiv.), PTSA, DMF, 70 °C; b) DIBAL-H (2.5 equiv.), CH_2Cl_2, –78 °C; 90 % yield for two steps; ii) (–)-(*S*)-camphanic chloride, pyridine, 0 °C to rt, **82** (80 % yield), **83** (82 % yield); iii) NaOH, MeOH, reflux, 94–97 %; iv) a) NaH, BnBr, DMF; b) CF_3COOH, CH_2Cl_2; 87 % yield for two steps.

3.4.4 Glycosidation and deprotection

An initial attempt at glycosidation with donor (**47**) and acceptor (**75**) used conditions reported by Y. Watanabe *et al.*[151] They detailed glycosidation conditions for phosphite donors where α-stereoselectivity is highly favoured, i.e. at room temperature in diethyl ether with zinc chloride (1.2 equivalents) and silver perchlorate (2.4 equivalents) as promoters and in the presence of 4Å molecular sieves. Observation of some clumping together of the molecular sieves in the reaction mixture after addition of the promoters seemed to suggest a low solubility of either the donor or acceptor (or both) in the reaction conditions, and subsequently the glycosidation gave disappointing results, with a low yield of an inseparable mixture of α- and β-coupled products. It was concluded that solubility of reactants was probably the key factor impeding a smooth reaction, thus another reaction solvent was sought.

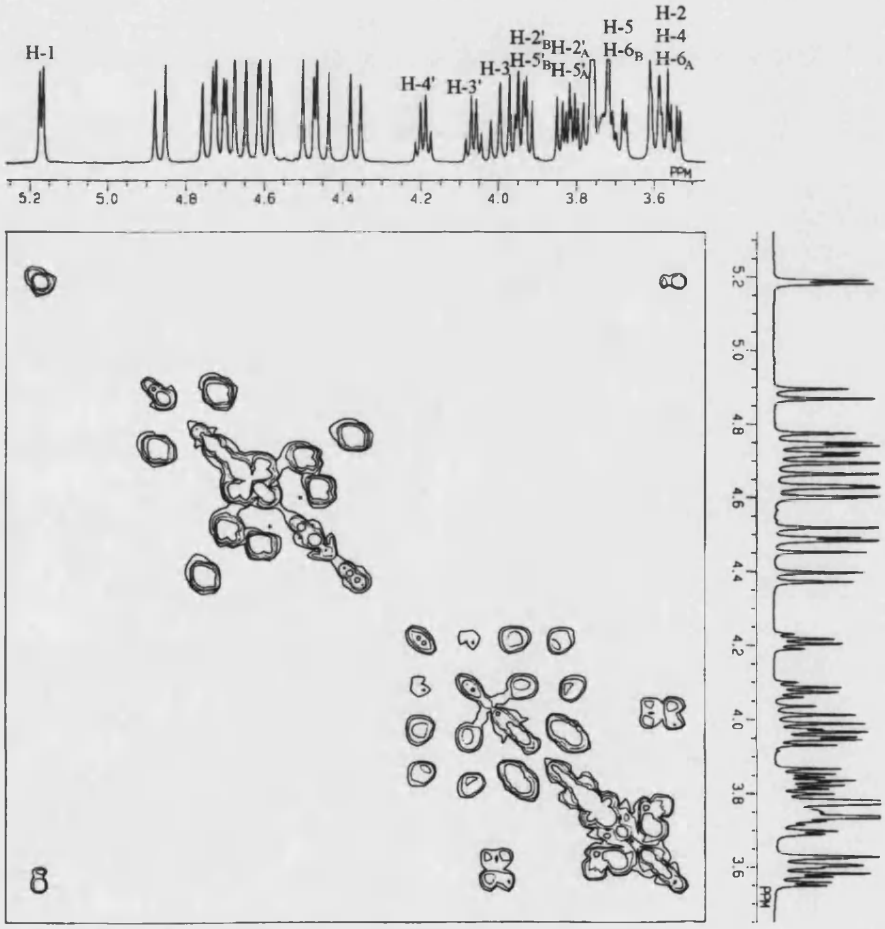


Figure **3.11**: Part of the ^{1}H-^{1}H COSY of **76**.

A recent publication[181] of iodonium-ion mediated glycosidation of thioglycosides in solvent mixtures with similar dipole moments and dielectric constants to diethyl ether found that a mixture of toluene/1,4-dioxane showed the greatest promise. Much higher α-selectivities with this solvent mixture were reported than with conventional glycosylation solvents, and a ratio of 1:4 toluene:dioxane was found to give the best results. This solvent mixture was therefore used in the place of diethyl ether in the phosphite coupling. The reaction mixture exhibited no signs of low solubility of the reactants and yielded solely the α-coupled product (**76**) in 76% yield. The ^{1}H NMR spectrum exhibited the characteristic resonance of a deshielded equatorial anomeric proton with small coupling constant (δ_H 5.18, J 3.4 Hz), typical of α-coupled sugars (Figure **3.11**).

Although deprotection of the *p*-methoxybenzyl groups of **76** in the synthesis of ribophostin was achieved successfully the yield was only moderate. New reaction conditions that cleave *p*-methoxybenzyl ethers have appeared recently in the literature, notably iodine in methanol,[182] magnesium bromide diethyl etherate-methyl sulfide[183] and trifluoroacetic acid in dichloromethane.[184] The latter conditions were chosen for the deprotection of **76** as they have been reported to selectively cleave *p*-methoxybenzyl ethers in the presence of multiple glycosidic linkages. The treatment of **76** with 10% trifluoroacetic acid in dichloromethane proved very effective and resulted in the desired triol (**77**) in high yield (84%).

3.4.5 Phosphorylation and Deprotection

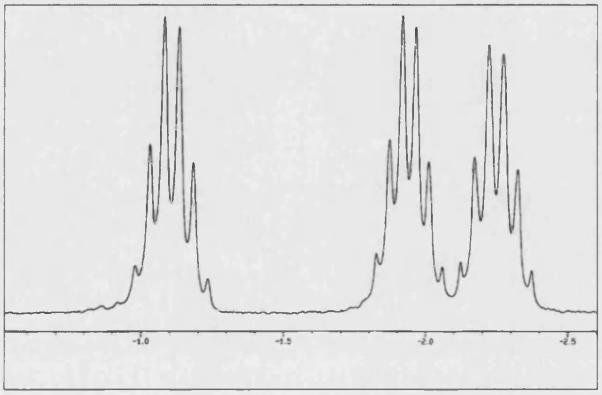


Figure **3.12**: ^{1}H-coupled ^{31}P NMR spectrum of **78**.

77 Was phosphitylated in the usual way with bis(benzyloxy) (diisopropylamino)phosphine activated with tetrazole to give a trisphosphite intermediate. This was oxidised with MCPBA to give the fully protected intermediate (**78**) in high yield. ^{31}P NMR confirmed the presence of three phosphate groups, shown in Figure **3.12**.

Catalytic hydrogenation with palladium on carbon in a mixture of water and methanol, followed by purification with ion exchange chromatography gave the completely deprotected target compound **79**, which was quantified by the Briggs phosphate assay.

3.5 Biological results

The biological testing by a collaborator with the same method used for adenophostin A indicated that furanophostin behaves as a full agonist, with potency in Ca^{2+} release assays in permeabilised hepatocytes similar to those of $Ins(1,4,5)P_3$ and ribophostin.

Maximally effective concentrations (10 μM) of either $Ins(1,4,5)P_3$ or furanophostin released the same fraction of the intracellular Ca^{2+} stores, 29 ± 6 % and 29 ± 1 % respectively. A similar response, release of 28 ± 2 % of the actively sequestered Ca^{2+}, was evoked by the simultaneous addition of maximal concentration of both agonists. The concentration of furanophostin required to cause half-maximal Ca^{2+} release (EC_{50}) was only 1.7-fold higher than that for $Ins(1,4,5)P_3$, and the responses to both agonists were positively co-operative (Table **3.3**).

	EC_{50} (nM)	h	n
$Ins(1,4,5)P_3$	194±30	2.08±0.22	3
Furanophostin	329±48	2.18±0.46	5

Table **3.3**. $^{45}Ca^{2+}$ release data for $Ins(1,4,5)P_3$ and furanophostin. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[180] Results are shown as means ± S.E.M.

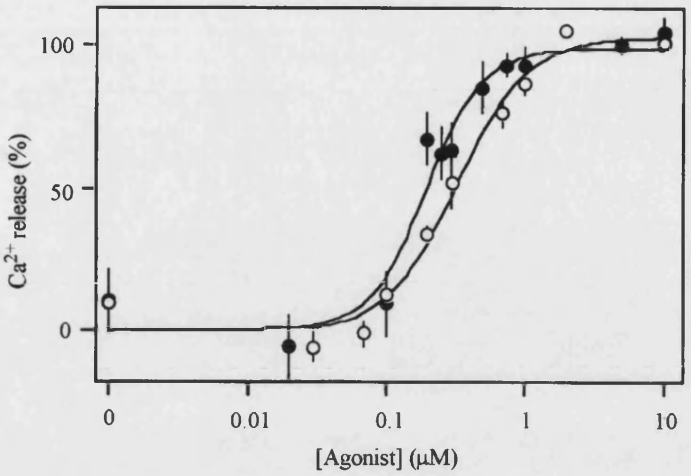


Figure **3.13**: $^{45}Ca^{2+}$ release from permeabilised hepatocytes by Ins(1,4,5)P_3 (●) and **7** (O). For each experiment, the $^{45}Ca^{2+}$ released in response to each concentration of agonist was determined and the concentration-effect relationship was then fitted to a logistic equation[180] from which the maximal response was determined. The results (means ± S.E.M. of 3–5 independent determinations) are shown as percentages of that maximal response.

3.6 Further minimal structure analogues

Several other minimal structure analogues have been reported since this work was commenced, including another synthesis of furanophostin.[185;186] This synthesis was accompanied by biological testing data for binding to porcine cerebella, indicating an IC_{50} value of 25nM, which is comparable to the affinity of Ins(1,4,5)P_3 itself in this assay (IC_{50} 19nM), thus confirming our Ca^{2+} release results that furanophostin is almost equipotent to Ins(1,4,5)P_3. Shuto *et al.* also reported the synthesis and biological testing of some related compounds shown in Figure **3.14**. Of particular interest is **85**, which has a structure midway between furanophostin and ribophostin: it contains a hydroxymethyl group analogous to the ribophostin 4-hydroxymethyl, but like furanophostin lacks the 1-*O*-methyl group. **85** Has an IC_{50} very similar to furanophostin, indicating that the 4-hydroxymethyl motif does not increase affinity for the Ins(1,4,5)P_3 receptor towards that of adenophostin A, and neither does it seem necessary for Ins(1,4,5)P_3-like activity.

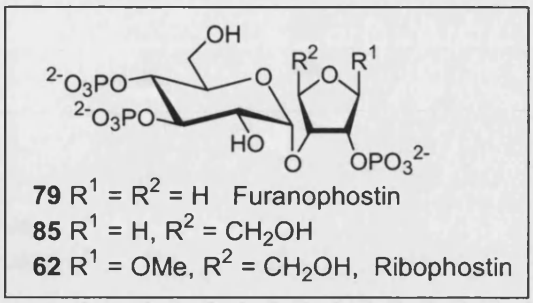


Figure **3.14**: Structures of three minimal structure analogues retaining the ribose five-membered ring.

Three further disaccharide adenophostin A analogues have also been reported, (Figure **3.15**) based on α,α'-trehalose and sucrose, both of which are readily available naturally occurring starting materials.[180] It was found that simple selective protection furnished intermediates suitable for phosphorylation to make target polyphosphates, all of which contained the same glucose-based structure as adenophostin A and placed the third phosphate on a second ring in a more rigid conformation that Gluc(2',3,4)P_3. Although molecular modeling indicated that the positioning of the third phosphate may not have been ideal, these were easily accessible analogues avoiding the need for glycosidation coupling reactions.

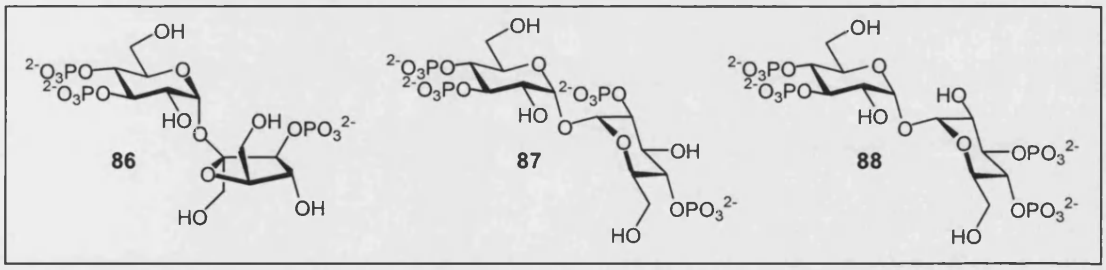


Figure **3.15**: Disaaccharide polyphosphates

The effects of these analogues, ribophostin and Gluc(2',3,4)P_3 on [^{3}H]Ins(1,4,5)P_3 binding and $^{45}Ca^{2+}$ mobilisation from rat hepatocytes which predominantly express type 2 Ins(1,4,5)P_3 receptors were recently compared.[180] Sucrose 3,4,3'-trisphosphate [Sucr(3,4,3')P_3] (**86**) was found to be 25-fold less potent in Ca^{2+} release than ribophostin, which is surprising as it is structurally quite similar to ribophostin. It was suggested that this was caused by one or all of the following factors: i). Steric hindrance from one or both of the hydroxymethyl groups on the fructofuranosyl ring. ii). Overlapping anomeric

effects about the glycosidic linkage, not present in ribophostin, influence the conformation about the glycosidic linkage. iii). The presence of a quaternary furanosyl anomeric center may lead to increased flexibility about the fructofuranosyl linkage.

Of the two α,α'-trehalose based analogues, the unsymmetrical α,α'-trehalose 2,4,3',4'-tetrakisphosphate [Trehal(2,4,3',4')P_4 (**87**)], gave the best results with a potency 10-fold lower than Ins(1,4,5)P_3, suggesting that the positioning of the 2'-phosphate group must be better than either of the 3' or 4'-phosphates of **88** (assuming that one glucose bisphosphate residue of **88** interacts with the bisphosphate recognition site and the other is left to interact with the accessory phosphate recognition site). It was also reasoned that the alteration in the substitution pattern on one glucose residue may influence the conformation about the glycosidic linkage.

Two further inositol based adenophostin A analogues (**89** and **90**) have been reported very recently[187] (Figure **3.16**) in an attempt to optimise the positioning of the non-vicinal phosphate group at the Ins(1,4,5)P_3 receptor and explore the possibility that correct positioning of this phosphate may engender adenophostin-like activity without the need for an adenosine structure. In both analogues this phosphate is positioned away from the inositol ring in a in a conformationally restrained manner. We are awaiting the biological evaluation of these compounds and therefore cannot yet comment on their structure-activity relationships.

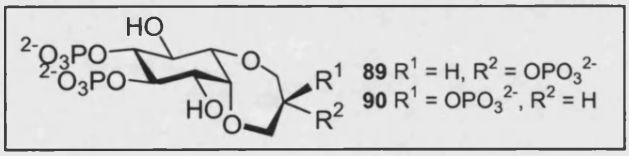


Figure **3.16**: Bicyclic analogues based upon adenophostin A.

Finally several clustered disaccharide polyphosphate analogues (**91**, **92** and **93**) have been prepared[188] (Figure **3.17**) in an effort to exploit the tetrameric nature of the Ins(1,4,5)P_3 receptor which has been shown to have four independent ligand binding sites. Although the biological evaluation of these analogues has not yet been reported it is unlikely that they will exhibited enhanced activity relative to ribophostin as the linking structure is not large enough to span the Ins(1,4,5)P_3 receptor.[189]

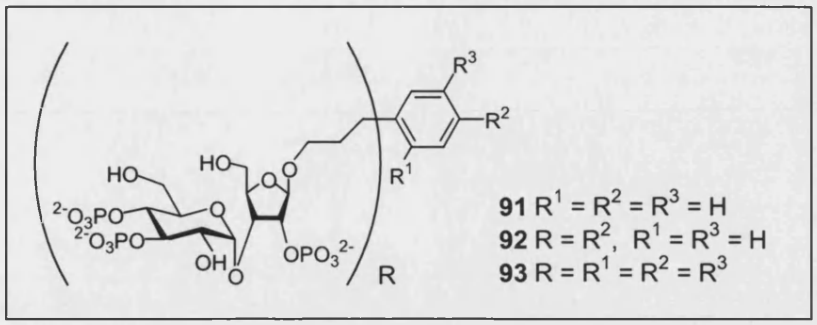


Figure **3.17**: Clustered disaccharide polyphosphate analogues of adenophostin A.

3.7 Summary

It is clear from the results published so far that the conformational restraint of the non-vicinal phosphate group analogous to the 2'-phosphate of adenophostin A is necessary for activity similar to Ins(1,4,5)P_3, since Gluc(2',3,4)P_3 is approximately 10-fold less potent than Ins(1,4,5)P_3. The more rigid five-membered ring common to ribophostin and furanophostin probably delivers their 2- and 3'-phosphates respectively to an optimal position for Ins(1,4,5)P_3-like activity. Although their activities were not compared directly in the same biological assay, comparison of binding results for ribophostin and **85** do indicate that while the 1-*O*-methyl of ribophostin does not seem to hinder activity, neither is it necessary for Ins(1,4,5)P_3-like activity. Similarly comparison of **85** and furanophostin indicates that the hydroxymethyl group is not essential for Ins(1,4,5)P_3-like activity, but it may yet be shown to play a role in the activity of adenophostin A because the interaction of adenophostin A with the receptor binding site must in some way be different to these minimal structure analogues.

Thus, furanophostin probably approaches the minimal structure for potent agonism in a simple carbohydrate-based polyphosphate mimic of adenophostin A. But from results published so far we can also conclude that the adenine component of the adenophostins plays a pivotal role in their activity presumably by interacting with a nearby binding pocket of the Ins(1,4,5)P_3 receptor and optimising binding interactions, and/or placing the 2'-phosphate in an optimal position at the binding site.

Chapter four

Sugar modified analogues

4 Sugar modified analogues

4.1 Introduction

The belief that the activity of the adenophostins stems from the phosphorylated glucose moiety is supported by the inactivity of 2'-AMP at releasing Ca^{2+} from intracellular stores of permeabilised platelets.[168] This is given further credence by the ability of minimal structure analogues based on glucose, and lacking adenosine, to release Ca^{2+}[180] (see chapter **3**). In addition the structure of the glucose moiety of adenophostin A most closely resembles that of Ins(1,4,5)P_3, and therefore some changes in the structure of Ins(1,4,5)P_3 and the subsequent effect on activity may be useful in predicting the biological effect of changes in the adenophostin A glucose ring.

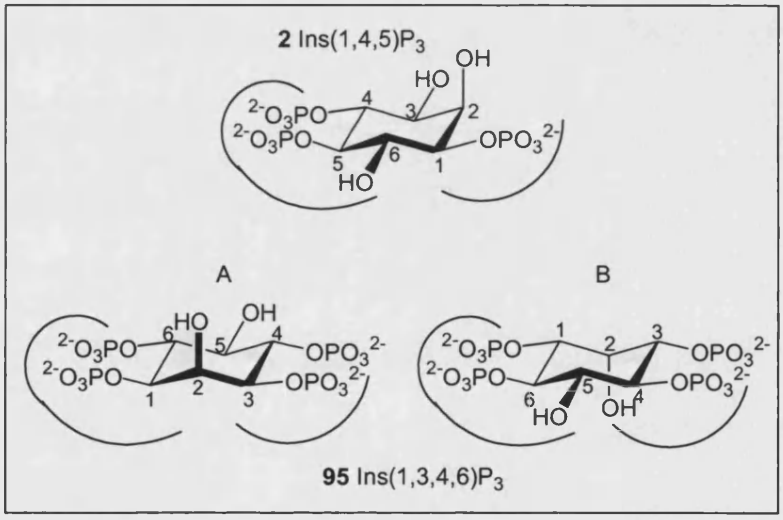


Figure **4.1**: Representation of the two possible binding orientations of Ins(1,3,4,6)P_4, A and B, at the Ins(1,4,5)P_3-binding site. (1D-numberings of the inositol ring are shown).

Biological evaluation of many synthetic Ins(1,4,5)P_3 analogues modified at one or several of the functional groups around the ring has brought greater understanding of the contribution they make to the activity of Ins(1,4,5)P_3. In particular the investigation into the binding orientation of the *meso* compound *myo*-inositol-1,3,4,6-tetrakisphosphate [Ins(1,3,4,6)P_4, **94**] and related molecules has brought an appreciation of the orientation of the hydroxyl groups of Ins(1,4,5)P_3 required for its binding and Ca^{2+} releasing activity.

The finding that Ins(1,3,4,6)P_4 was capable of releasing Ca^{2+} from the intracellular stores of permeabilised rabbit platelets, but was 40-fold less potent than Ins(1,4,5)P_3[190] led to a rationalisation of its activity with two different binding orientations. This required the vicinal D-1,6-bisphosphate of Ins(1,3,4,6)P_4 to mimic the Ins(1,4,5)P_3 D-4,5-bisphosphate, and placed the axial 2-hydroxyl of Ins(1,3,4,6)P_4 in a position equivalent to the 3 or 6-position of Ins(1,4,5)P_3, (see Figure **4.2**).

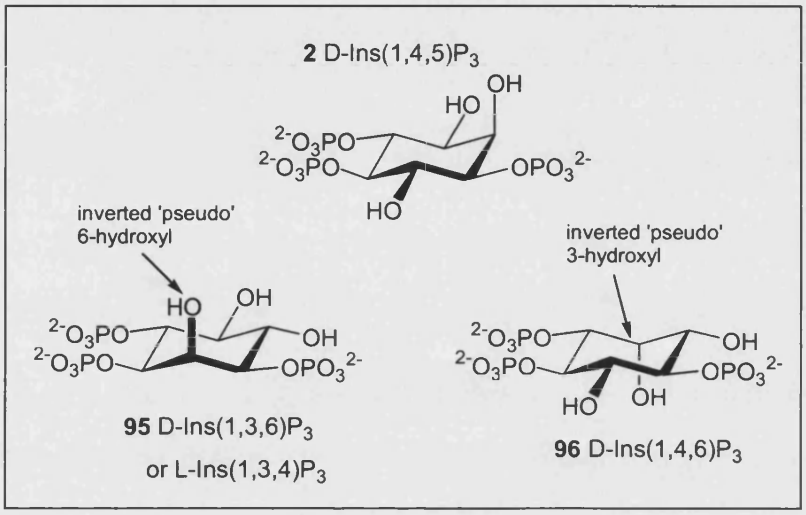


Figure **4.2**: Representation of L-Ins(1,3,4)P_3 and D-Ins(1,4,6)P_3 to show the inverted 6 and 3-hydroxyls in a binding orientation similar to Ins(1,4,5)P_3.

It was therefore hypothesised that D-*myo*-inositol-1,4,6-trisphosphate [D-Ins(1,4,6)P_3, **96**] and D-*myo*-inositol-1,3,6-trisphosphate [D-Ins(1,3,6)P_3, **95**], analogues in which the 3 or 6-hydroxyl had been inverted respectively, would possess Ca^{2+} releasing activity. Though they were strictly analogues of *scyllo*-Ins(1,4,5)P_3 (because the pseudo 2-hydroxyl was equatorial rather than axial), this was considered to be of little consequence because DL-*scyllo*-Ins(1,2,4)P_3[191] and DL-2-deoxy-Ins(1,4,5)P_3[167] have been shown to be only approximately 2.5-fold weaker than Ins(1,4,5)P_3 in biological activity. (A *chiro*-inositol based analogue of Ins(1,4,5)P_3, L-*chiro*-inositol 2,3,5-trisphosphate was previously synthesised. This is an analogue of Ins(1,4,5)P_3 in which the 3-hydroxyl was inverted and the 2-hydroxyl remained axial with potency approximately 10-fold lower than Ins(1,4,5)P_3 in permeabilised SH-SY5Y cells[192]).

Hence the total synthesis and biological evaluation of D-Ins(1,4,6)P_3 and D-Ins(1,3,6)P_3 [alternative nomenclature: L-Ins(1,3,4)P_3] showed that these analogues were indeed active,[190] with D-Ins(1,4,6)P_3 (in which the 3-hydroxyl was axial) being only 2–3-fold less potent than Ins(1,4,5)P_3, and L-Ins(1,3,4)P_3 (in which the 6-hydroxyl was axial)

being 12-fold less potent than Ins(1,4,5)P_3. Furthermore, Ins(1,4,5)P_3 analogues previously reported in the literature where the hydroxyls at positions 3 and 6 were deleted have been biologically evaluated for Ca^{2+} release. The results indicated that although both were active, the absence of the 6-hydroxyl resulted in a greater loss of activity[193] than the absence of the 3-hydroxyl.[194]

Comparison of the structure of these Ins(1,4,5)P_3 analogues modified at the 3 and 6-positions with the adenophostin A glucopyranose ring structure quickly brings to light the possibility of modification at the analogous positions in adenophostin A (Figure **4.3**). Since the 3",4"-bisphosphate and the adjacent 2"-hydroxyl of adenophostin A possess identical relative stereochemistry to the 4,5-bisphosphate and adjacent 6-hydroxyl of Ins(1,4,5)P_3 it follows that the 2" and 5" positions of adenophostin A may be considered analogous to the 6 and 3 positions of Ins(1,4,5)P_3 respectively. Therefore, with the above discussion in mind it is possible to speculate on the effect of modification of the 2" and 5" positions of the glucose ring and identify suitable sugar modified analogue targets of adenophostin A.

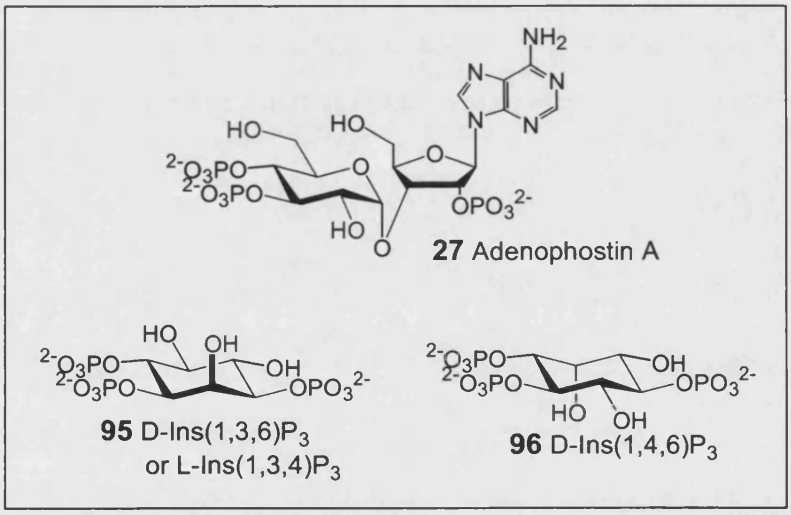


Figure **4.3**: Comparison of Ins(1,4,5)P_3 analogues modified at positions 3 and 6 with that of adenophostin A.

Working within the constraints of readily available monosaccharide starting materials, two sugar modified adenophostin A analogue targets were selected (see Figure **4.4**). The first was based on mannose, which has an axial hydroxyl at its 2-position. This epimerisation with respect to adenophostin A is equivalent to having an axial phosphate at the 6-position of Ins(1,4,5)P_3. Therefore D-Ins(1,3,6)P_3 [L-Ins(1,3,4)P_3] may be thought of as the closest Ins(1,4,5)P_3 analogue counterpart of the mannose

analogue of adenophostin A, although the mannose analogue has a hydroxymethyl group in a position equivalent to the 3-hydroxyl of Ins(1,4,5)P_3. Bearing this in mind, the activity of a mannose adenophostin A analogue might be expected to be considerably lower than that of adenophostin A

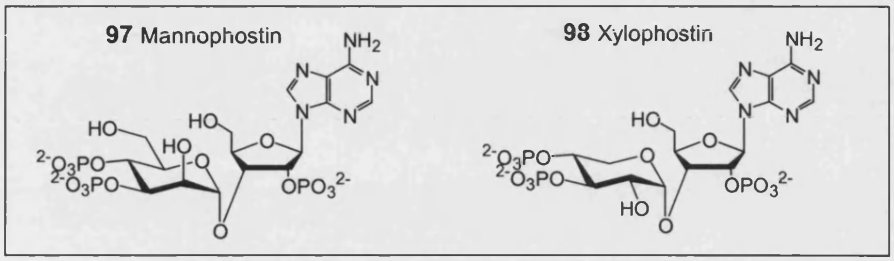


Figure **4.4**: The structures of mannophostin and xylophostin.

It was decided that a second sugar analogue would be based on xylose, because a sugar with an inverted hydroxyl at position 6 was not readily accessible. Such an adenophostin A analogue lacks the 5"-hydroxymethyl, the position corresponding to the Ins(1,4,5)P_3 3-hydroxyl. As outlined above 3-modified analogues of Ins(1,4,5)P_3 have already been made with both 3-deoxy Ins(1,4,5)P_3 and D-Ins(1,4,6)P_3 being slightly weaker than Ins(1,4,5)P_3. It might be predicted that the xylose based adenophostin A analogue would have slightly lower activity than adenophostin A itself, but, be more active than Ins(1,4,5)P_3. It is also interesting to note that 3-deoxy Ins(1,4,5)P_3 appears to be a type II partial agonist[99] (see also chapter one) and the phosphorothioates of D-Ins(1,4,6)P_3 and D-Ins(1,3,6)P_3 have also been defined as type I partial agonists. Therefore the xylophostin and mannophostin equivalents, with their (presumably) enhanced affinity for the receptor, may also exhibit interesting partial agonist activity.

A convergent approach to the synthesis to these molecules was adopted, using the adenosine acceptor described in chapter **2** for the synthesis of adenophostin A and suitably protected mannose and xylose donors.

4.2 Synthesis of Mannophostin

4.2.1 Synthesis of the donor

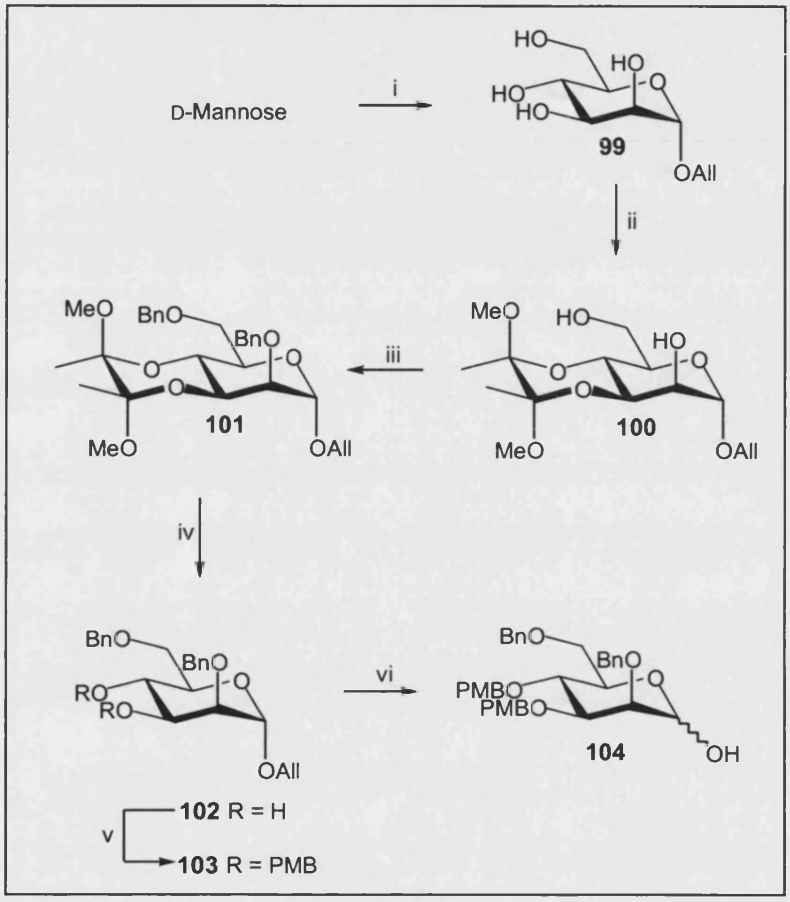


Figure **4.5**: Route to fully protected D-mannopyranose **104**.

Reagents and conditions: i) AllOH, acetyl chloride, reflux; ii) $MeC(OMe)_2C(OMe)_2Me$, CSA, MeOH, $(MeO)_3CH$, reflux; iii) NaH, BnBr, DMF; iv) 95% aq. TFA-DCM (1:1); v) NaH, PMBCl, DMF; vi) $PdCl_2$, MeOH.

The fully protected D-mannopyranose suitable for conversion to the mannose phosphite donor was prepared by a colleague (A. M. Riley) as shown in Figure **4.5**. Although intermediate **101** with the butane diacetal (BDA) still in place may appear to be a suitable precursor to a mannose-based donor it has been reported that protection with a BDA group has a considerable deactivating effect. It was suggested that this effect may be due to the rigidity of such protection hindering the flattening of the sugar ring which occurs during oxonium ion formation.[195] Therefore, the BDA group was

removed and replaced with *p*-methoxybenzyl ethers. Conversion into the dimethyl phosphite was achieved by treatment of **104** with tetrazole and bis(methoxy)(diethylamino)phosphine in DCM. The ^{31}P and ^{1}H NMR spectra of the phosphitylated product (**105**) indicated that the α-anomer had almost exclusively been formed. The characteristically deshielded anomeric proton of the α-anomer observed at δ_H 5.52 exhibited both a small equatorial-equatorial coupling to H-2 (J 1.8 Hz) and a large coupling to the phosphite phosphorus atom ($J_{H\text{-}P}$ 8.2 Hz). Furthermore, the slightly more upfield ^{31}P signal from the α-anomer was observed in the ^{31}P NMR spectrum (Figure **4.6**). The α-configuration at this position is preferentially formed as there are unfavourable electrostatic interactions between the axial 2-position and the equatorial β-anomer 1- position. In addition the α-anomeric effect favours the α-configuration at the anomeric position.

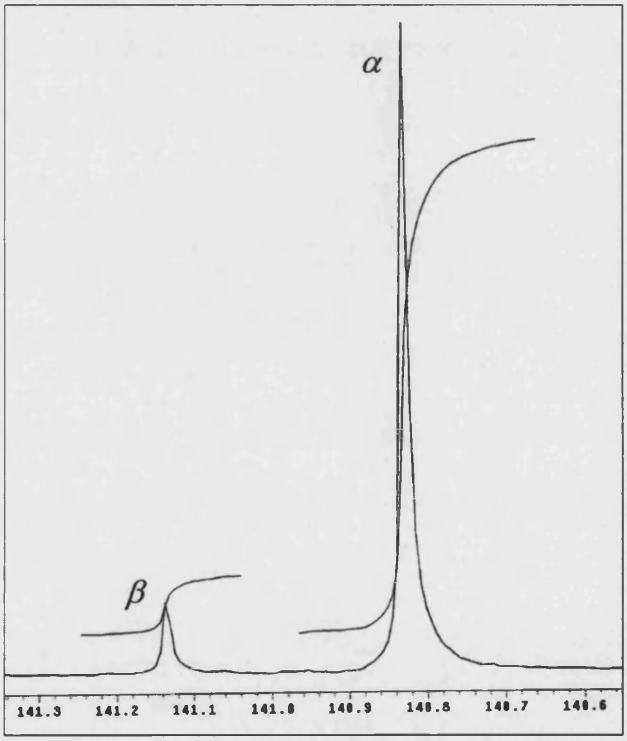


Figure **4.6**: ^{31}P NMR spectrum of **104**.

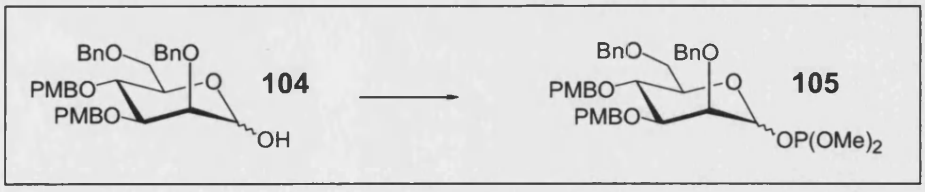


Figure **4.7**: Conversion of **104** into a dimethyl phosphite.

4.2.2 Glycosidation and deprotection

The adenosine donor prepared for the synthesis of adenophostin A was coupled to the mannose phosphite donor with zinc chloride and silver perchlorate as promoters in a similar fashion to that used in the synthesis of adenophostin A. The reaction went smoothly in 8 hours to furnish the fully protected intermediate **106** in 61% yield. This isolated product was found to be solely the α-coupled compound by examination of the ^{1}H NMR spectrum which exhibited a deshielded singlet at δ_H 5.05 corresponding to H-1".

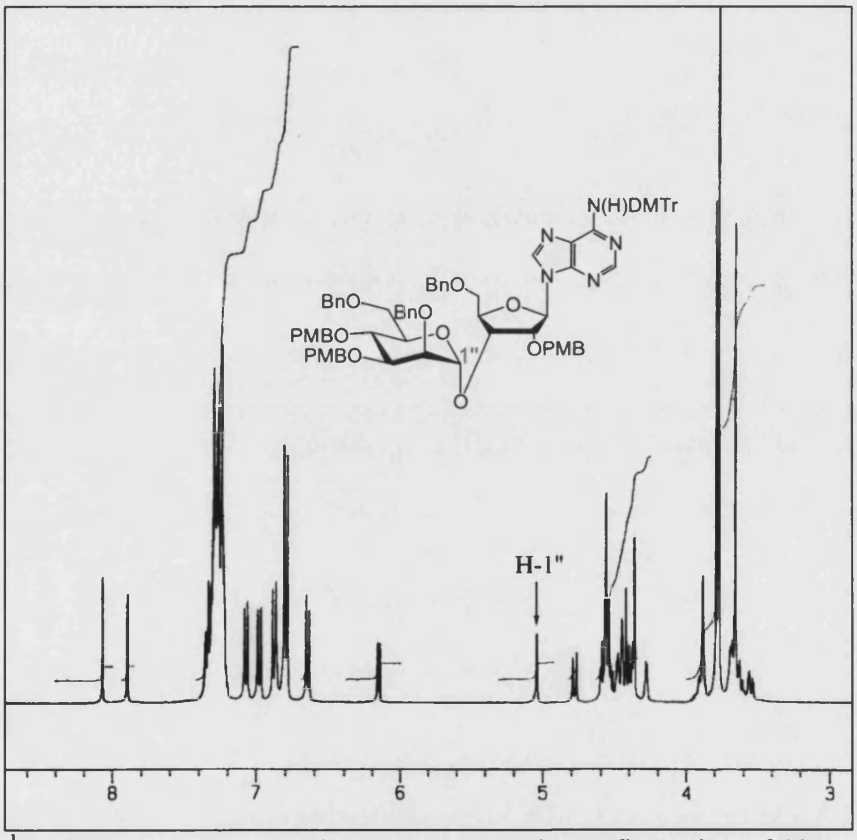


Figure **4.8**: ^{1}H NMR spectrum showing the α-anomeric configuration of **106**.

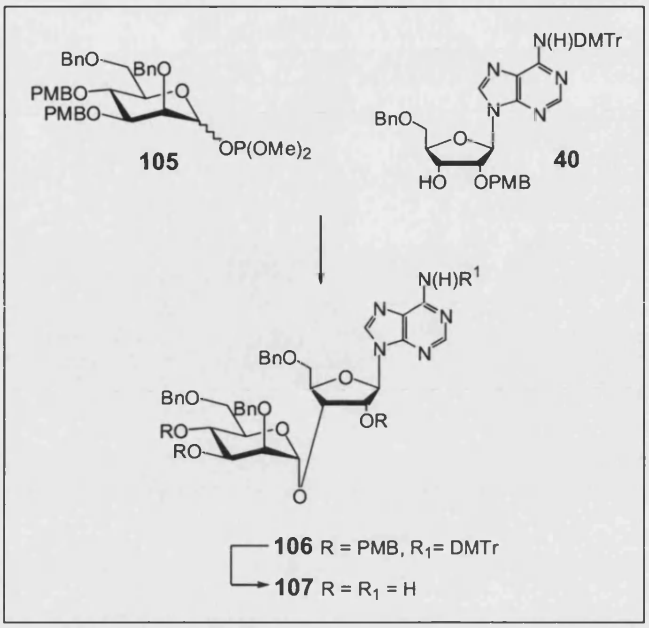


Figure **4.9**: Glycosidation and deprotection

Cleavage of the all the acid-labile protecting groups was achieved with 10% TFA in DCM. The equivalent reaction in the synthesis of adenophostin A only took 1.75 hours, while complete deprotection for the mannose-based intermediate took 5 hours. Monitoring of the reaction by TLC indicated that cleavage of the DMTr group was quickly complete, and that it was probably one of the *p*-methoxybenzyl groups that was more refractory to the reaction conditions. It is most likely that the *p*-methoxybenzyl group concerned was that at the 2'-position, and that the α-D-mannopyranoside affected the conformation of the ribofuranose ring in such a way as to increase the π-stacking interaction between the phenyl ring of the 2'-*O*-*p*-methoxybenzyl group and the adenine base.

The resulting triol (**107**) was found to be barely soluble in most of the common organic solvents and so the NMR spectra were accumulated from a sample in deuterated DMF. The absence of the acid-labile protecting groups was obvious from the ^{1}H NMR spectrum where the aromatic region was occupied by signals from the three remaining benzyl groups. In addition the signal from H-1" was still present at δ_H 5.37, indicating that extended exposure to 10% TFA in DCM had not been detrimental to the glycosidic or nucleosidic linkages.

4.2.3 Phosphorylation and Deprotection

The triol was phosphorylated using the same phosphitylating reagents described for the phosphitylation of the corresponding adenophostin A triol. Again there was no need for protection of the N^6-position prior to phosphitylation. Subsequent oxidation with MCPBA furnished the fully protected trisphosphate, identified by the three sextets in the coupled ^{31}P NMR spectrum.

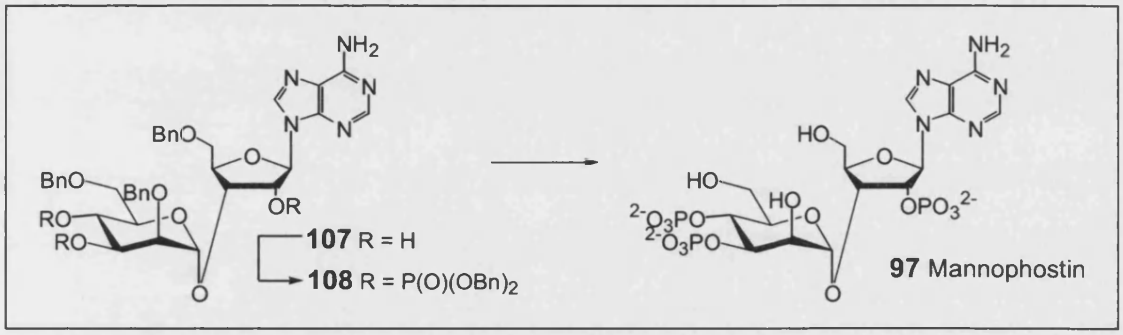


Figure **4.10**: Selective phosphorylation and deprotection of **107** to give mannophostin

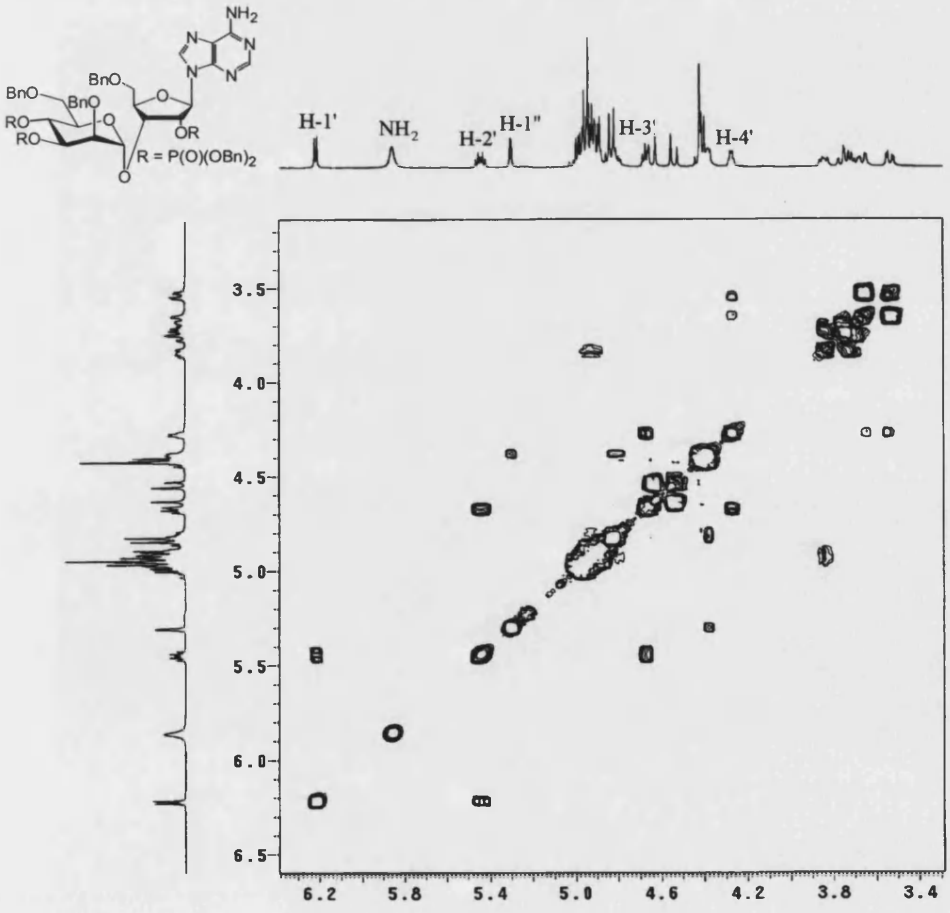


Figure **4.11**: Part of the ^1H-^1H COSY of **108** indicating the free N^6 position.

Complete deprotection was achieved with catalytic transfer hydrogenation conditions. Thus treatment with 20% palladium hydroxide on carbon in a mixture of cyclohexane, methanol and water for 10 hours gave the free acid of the desired mannose analogue of adenophostin A **97** (mannophostin). Unfortunately the ^{31}P and ^{1}H NMR spectra clearly showed a minor impurity, probably the migration product of ring-opening of a cyclic intermediate. Formation of such an intermediate is favoured by the *cis*-relationship of positions 2 and 3 on the mannose ring. Thus the reaction was repeated for a shorter length of time (2.5 hours), whereupon the ^{31}P and ^{1}H NMR spectra of the product purified by ion exchange indicated pure **97** in good yield. Finally the free acid was converted into its sodium salt by elution through a sodium ion exchange column, and quantified by both UV and Briggs total phosphate assay, which were in agreement.

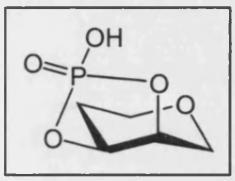


Figure **4.12**: Cyclic phosphate intermediate responsible for phosphate migration.

A sample of mannophostin was examined by HPLC using the same reverse phase column and method as those used in the analysis of the adenophostin A samples in chapter **2**; the resulting trace is shown in Figure **4.13**.

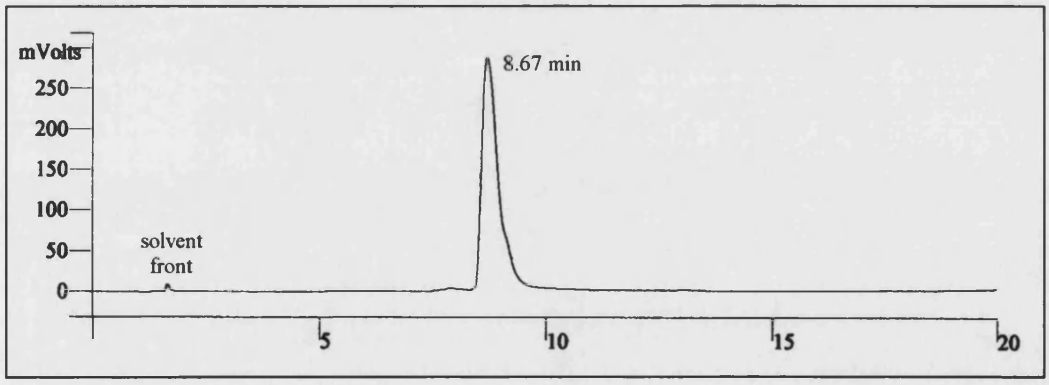


Figure **4.13**: HPLC trace of mannophostin.

4.3 Biological results

Mannophostin was tested by a collaborator, with the same method used to test adenophostin A, for Ca^{2+} mobilisation in permeabilised hepatocytes and the results are shown in Table **4.1** with results for Ins(1,4,5)P_3 and adenophostin A. The activity of mannophostin was clearly weaker than both that of adenophostin A and Ins(1,4,5)P_3.

	EC_{50} (nM)	*h*	Maximal response %	n
Mannophostin	180±16	2.85±0.55	49±3	8
Ins(1,4,5)P_3	119±8	2.69±0.55	55±3	15
Adenophostin A	14±2	1.99±0.18	51±6	6

Table **4.1**. $^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3, adenophostin A and mannophostin. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation[180]. Results are shown as means ± S.E.M.

4.4 Synthesis of xylophostin

4.4.1 Synthesis of the donor

Although the required regioselectively protected D-xylopyranose **115** had already been prepared and reported by a colleague[88] (Figure **4.14**), the overall yield was restricted to less than 46% from allyl α-D-xylopyranoside by the unavoidable formation of two BDA regioisomers part-way though the synthesis.

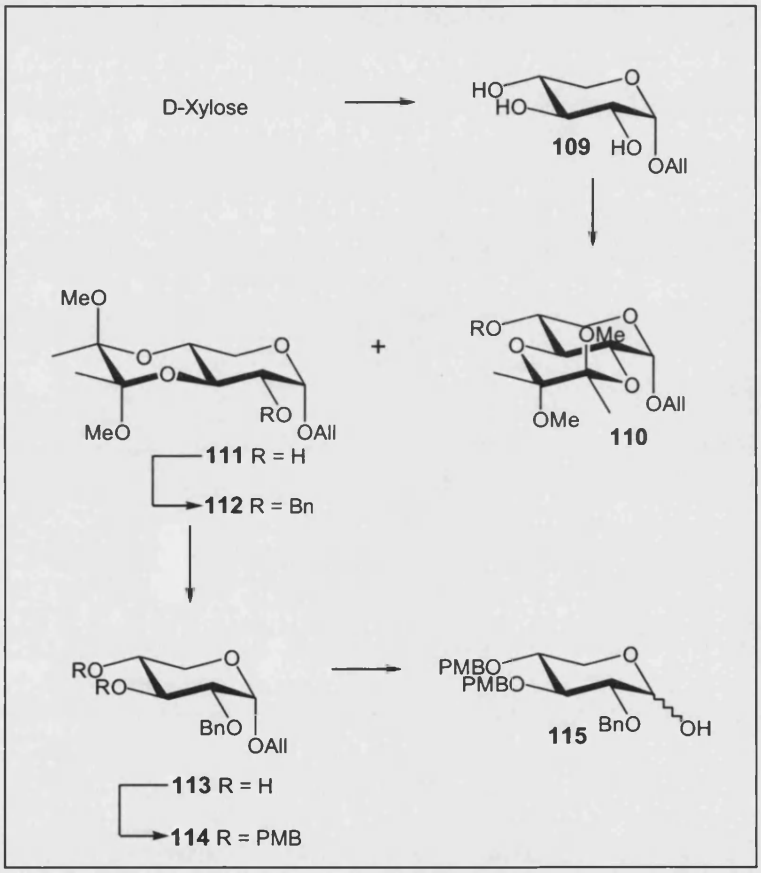


Figure **4.14**: Literature route to fully protected D-xylopyranose **115** developed by our group.

A different route was therefore investigated. Even though this proposed route was longer it was hoped that the overall yield would be higher and require less time-consuming purification by chromatography. Thus Fischer glycosidation by a slight modification of the method described by Jenkins & Potter[88]gave the allyl glycoside (**109**) as a fluffy white crystals in 41% yield over three crops. It was found that the reaction

mixture could be satisfactorily neutralised by addition of solid $NaHCO_3$, avoiding the need for more expensive Amberlite IR-45 (OH^-) resin. Furthermore adding diisopropyl ether to the crystallisation mixture immediately before filtration facilitated the isolation of the crystalline product and consequently the yield was significantly improved from that reported of 28%.

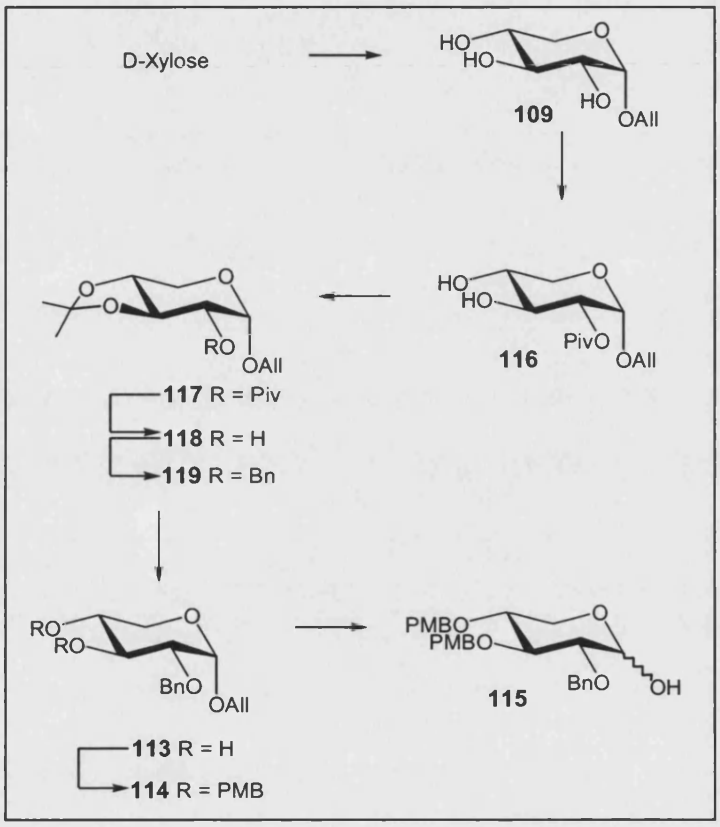


Figure **4.15**: Alternative route to fully protected D-xylopyranose **115**.

A modification of a selective pivaloylation method described in the literature[196] was used to selectively acylate the 2-hydroxyl of **109**. This particular report was concerned with regioselective acylation of a selection of carbohydrates, and although they did not actually attempt the reaction with a xylopyranoside treatment of methyl α-D-glucopyranoside was shown to give the 2,6-di-*O*-pivaloyl derivative in high yield (83%). Consequently, since the 5-hydroxymethyl is absent in xylose it was thought that there would be regioselectivity for the 2-hydroxyl in an analogous reaction. Treatment of **109** with pivaloyl chloride at –20°C in pyridine did indeed give one major product, although other minor products were shown to be present by TLC. Since a colleague (H. Rosenberg) carried out this reaction at a similar time and identified the major product to

be the 2-*O*-pivaloyl derivative from the ^{1}H-^{1}H COSY NMR spectrum (which indicated a typically deshielded acylated doublet of doublets at δ_H 4.61 with coupling constants *J* 3.8 Hz and 10.0 Hz, corresponding to H-2), the crude material obtained from the reaction was carried forward to the next step.

The protection of the remaining *trans*-diequatorial hydroxyl groups at positions 3 and 4 was achieved by treating the crude residue with 2-methoxypropene and catalytic *p*TSA in dry THF to give one major product (**117**), which after purification was identified as the 3,4-*O*-isopropylidene derivative.[197]

Conversion into the known allyl 2-*O*-benzyl-α-D-xylopyranoside was then straightforward, with each intermediate being isolated for identification and yield purposes. The 2-*O*-pivaloyl was saponified by heating at reflux with NaOH pellets in methanol. The resulting free 2-hydroxyl was alkylated with benzyl bromide and sodium hydride in DMF, and the 3,4-*O*-isopropylidene easily removed by treatment of **119** with 10% 1M aqueous HCl in methanol. As these three steps were all found to be clean and high yielding reactions it was decided that the route up to **113** was suitable for large scale synthesis with just one purification of **113** by flash chromatography. Therefore the five steps from D-xylose to **113** were carried out on a 20g scale resulting in an overall yield of 60%. This was considered a significant improvement on the yield reported for the literature route from the same starting material to **113** of 39%.

The introduction of *p*-methoxybenzyl ethers at positions 3 and 4 was accomplished by treatment of **113** with sodium hydride and *p*-methoxybenzyl chloride in DMF at room temperature. The literature method for this step remarks that heating of the reaction mixture was necessary to give a good yield. It was therefore decided that the effect of two equivalents of each reagent per hydroxyl should be explored in an effort to increase the yield and avoid heating the reaction. This approach was successful and the fully protected intermediate (**114**) was isolated in 84% yield (literature 74%).

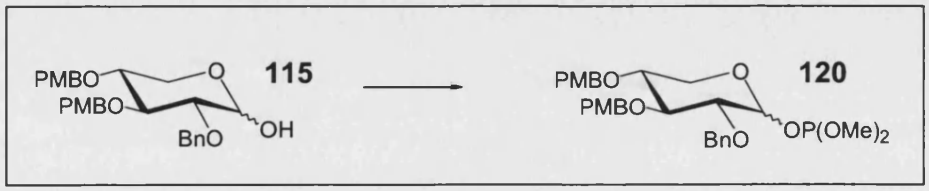


Figure **4.16**: Conversion of **115** into a dimethyl phosphite.

The allyl group at the anomeric position was cleaved with palladium chloride in methanol[198] to give the desired xylopyranose (**115**) in 90% yield as a 7:3 α:β anomeric mixture as judged from the ^{1}H NMR spectrum. This method represents a more convenient and higher yielding cleavage of the allyl glycoside than that reported in the literature. Finally **115** was converted into the phosphite donor (**120**) with tetrazole and bis(methoxy)(diethylamino) phosphine in DCM. The ^{31}P and ^{1}H NMR spectra of the phosphitylated product indicated a 2:3 α:β anomeric mixture as judged from the ^{1}H and ^{31}P NMR spectra.

4.4.2 Glycosidation and deprotection

The adenosine based acceptor **40** was glycosylated with the xylopyranoside phosphite donor **115** in the presence of zinc chloride and silver perchlorate in a similar manner as that previously described in the synthesis of adenophostin A. TLC of the reaction mixture indicated the formation of a single product, but ^{1}H NMR of the isolated material indicated that both α and β anomeric products (**121ab**) had formed in a 1:1 anomeric mixture (46%), with the signals for H-1' of the two anomers being immediately noticeable at δ_H 6.13 and δ_H 6.22. This anomeric mixture was found to be inseparable at this point and so it was treated with 10% TFA in DCM. The resulting two products (**122ab**) were easily separated by TLC, and were isolated after repeated purification by flash column chromatography.

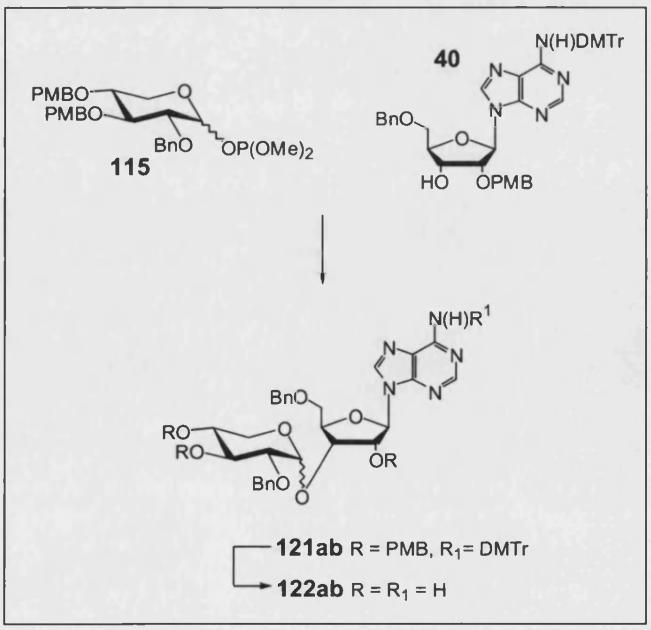


Figure **4.17**: Glycosidation and deprotection.

^{1}H NMR of these two triol anomers confirmed the configurations at the two anomeric centers. The more deshielded equatorial H-1" of the α-anomer (**122a**) was found to resonate at δ_H 5.17 with a small coupling constant of J 3.8 Hz while the more shielded axial H-1" for the β-anomer (**122b**) resonated at δ_H 4.46 with a larger coupling constant of J 7.9 Hz.

4.4.3 Phosphorylation and Deprotection

The α-coupled triol (**122a**) was successfully phosphitylated with imidazolium triflate and bis(benzyloxy)(diisopropylamino)phosphine in DCM without prior protection of the N^6 position. Oxidation with MCPBA gave, after purification, the fully protected xylose analogue intermediate **123**.

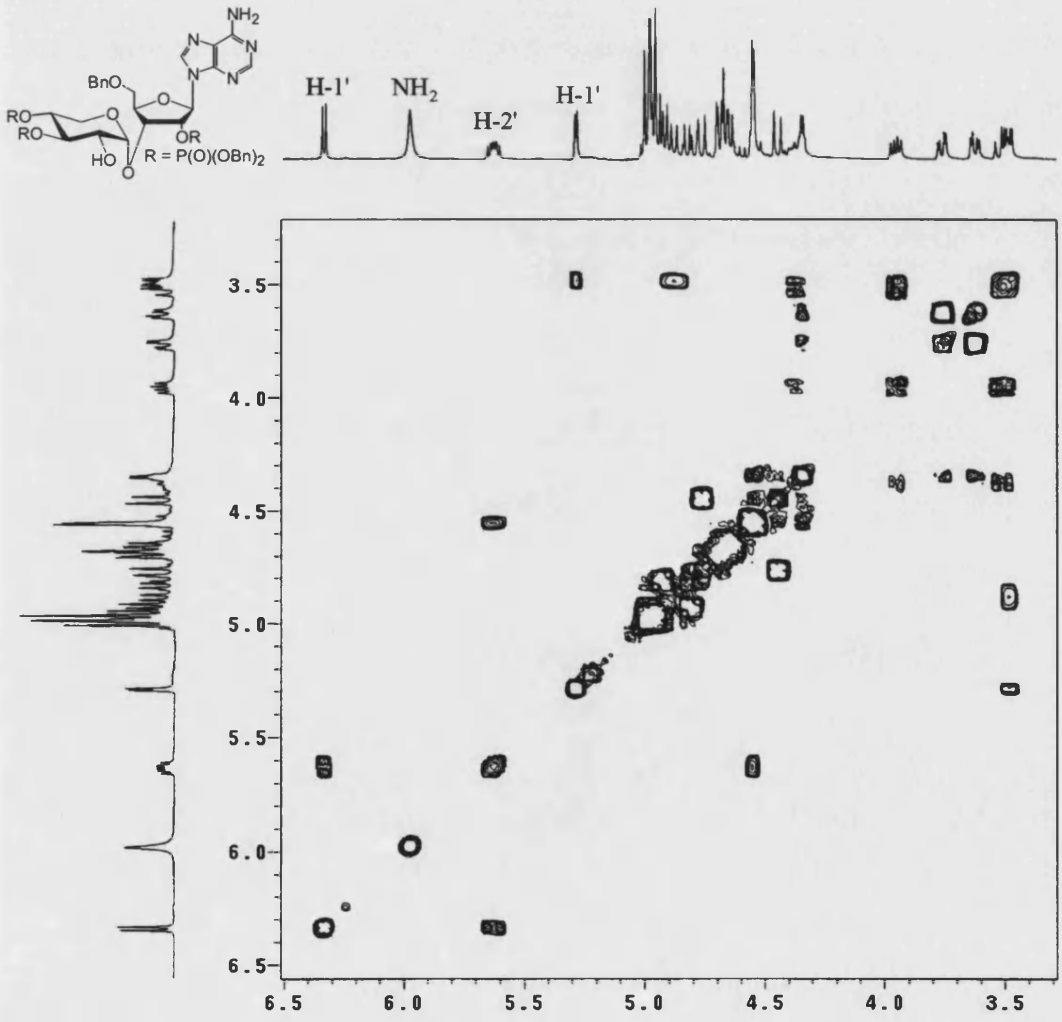


Figure **4.18**: Part of the ^{1}H-^{1}H COSY of **123** indicating the free N^6 position.

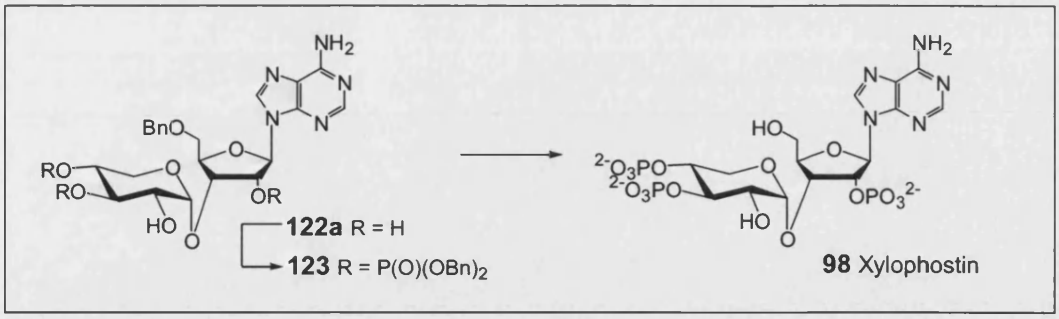


Figure **4.19**: Selective phosphorylation and deprotection of **122a** to give xylophostin

Complete cleavage of all the benzyl protecting groups of **123** was achieved with catalytic transfer hydrogenation, by treatment of **123** with 20 % palladium hydroxide on carbon in a mixture of cyclohexene, methanol and water. Purification by ion exchange chromatography with a 0–100 % gradient of 150 mM TFA as eluent to gave the xylose-based adenophostin A analogue xylophostin in good yield. Deprotection of **123** was confirmed by the ^{1}H NMR spectrum which showed an absence of benzyl protons, and the presence of a peak at *m/z* 638 of the negative ion FAB mass spectrum corresponding to $(M-1)^{-}$ of xylophostin.

The isolated free acid was converted into its sodium salt by elution through a sodium ion exchange column and finally quantified by UV and Briggs phosphate assay, both of which were in agreement.

A sample of xylophostin was also examined by HPLC with the same conditions and column used for the analysis of adenophostin A and the resulting trace is shown below in Figure **4.20**.

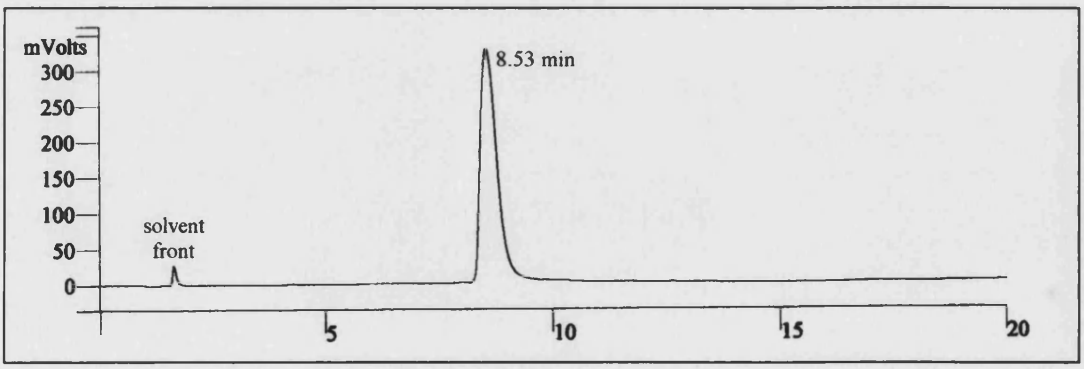


Figure **4.20**: HPLC trace of xylophostin.

4.5 Biological results

Xylophostin was tested by a collaborator for Ca^{2+} mobilisation in permeabilised hepatocytes with the method used in chapter **2** for adenophostin A. The results are shown in Table **4.2** with those for Ins(1,4,5)P_3 and adenophostin A. Xylophostin was found to be 2-fold weaker the adenophostin A in Ca^{2+} mobilisation from permeabilised hepatocytes

	EC_{50} (nM)	*h*	Maximal response %	n
Xylophostin	28±8	2.50±0.77	52±4	8
Ins(1,4,5)P_3	119±8	2.69±0.55	55±3	15
Adenophostin A	14±2	1.99±0.18	51±6	6

Table **4.2**. $^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3, adenophostin A and xylophostin. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[180] Results are shown as means ± S.E.M.

4.6 Summary

The biological data for mannophostin and xylophostin show that a change in the 2"-position of adenophostin A, has a far greater effect on its Ca^{2+} releasing ability compared with a change at the 5"-position. Previous discussion considered the current understanding on the alteration of the Ins(1,4,5)P_3 structure at positions 6 and 3, with the conclusion that modification at position 6 of Ins(1,4,5)P_3 has a more profound effect on its activity compared with modification at position 3. The Ca^{2+} releasing abilities of mannophostin and xylophostin therefore provide evidence for a binding orientation at the Ins(1,4,5)P_3 receptor, in which the 2"-hydroxyl and 5"-hydroxymethyl of adenophostin A mimic the 6-hydroxyl and 3-hydroxyl of Ins(1,4,5)P_3 respectively. Moreover, a more detailed comparison of the EC_{50} values given here for xylophostin and adenophostin A indicated that the deletion of the adenophostin A motif equivalent to the Ins(1,4,5)P_3 3-hydroxyl resulted in a 2-fold drop in activity. A similar drop in activity was observed

between 3-deoxy Ins(1,4,5)P_3 and Ins(1,4,5)P_3 in permeabilised SH-SY5Y cells, and between D-Ins(1,4,6)P_3 (**96**) (which has an axial pseudo 3-hydroxyl) and Ins(1,4,5)P_3 in permeabilised platelets. Comparison of the EC_{50} values for mannophostin, in which the position equivalent to the 6-hydroxyl of Ins(1,4,5)P_3 had been inverted, and adenophostin A found there was a 12-fold drop in activity. Interestingly, this was analogous to the 12-fold difference in activity between L-Ins(1,3,4)P_3 (**95**) (which has an axial pseudo 6-hydroxyl), discussed in section **4.1**, and Ins(1,4,5)P_3 when tested in permeabilised platelets. These observations therefore suggest that the introduction of the adenophostin A 2'-AMP motif to a monosaccharide version of an Ins(1,4,5)P_3 analogue improves the potency in a manner which correlates to the activity this Ins(1,4,5)P_3 analogue. With this in mind it may be possible to design sugar modified analogues containing the 2'-AMP motif for which the activity may be more accurately predicted.

In addition it is interesting that the deletion of the 5"-position of adenophostin A, found in xylophostin, and the resulting reduction in activity supports the findings from earlier studies (discussed in chapter **3**), that suggested that the activities of Gluc(2'3,4)P_3 (**58**) and its xylose equivalent were of a similar order.

Further biological investigation into the binding properties of mannophostin and xylophostin at the Ins(1,4,5)P_3 receptor is required as they might be partial agonists. Examination of the properties of the Ins(1,4,5)P_3 analogue analogous to xylophostin, 3-deoxy-*myo*-inositol 1,4,5-trisphosphate, led to the finding that it is a type II partial agonist[99](see chapter **1**). It can therefore be reasoned that xylophostin might be a partial agonists with amplified affinity for the Ins(1,4,5)P_3 receptor.

Moreover the synthesis of trisphosphorothioates of two Ins(1,4,5)P_3 analogues modified at the 3 and 6 positions has led to the synthesis of two type I partial agonists, DL-Ins(1,3,4)PS_3 and DL-Ins(1,4,6)PS_3. It is therefore possible that phosphorothioate analogues of mannophostin and xylophostin might also be type I partial agonists, but with increased affinity for the Ins(1,4,5)P_3 receptor.

Chapter five

Base modified analogues

5 Base modified analogues

5.1 Introduction

As previously discussed in chapter **3** the biological activity of the minimal structure analogues synthesized so far has not exceeded that of Ins(1,4,5)P_3. It would seem that activity in the realm of the adenophostins at least requires the adenine base or a similar motif. In an endeavour to ascertain the features of the adenine base crucial for adenophostin A-like activity the synthesis of two base modified analogues was planned based on purine and imidazole. It is clear that purine represents only a small modification of the adenine base with the absence of the N^6 amino group. The imidazole analogue, meanwhile, represented a much more drastically pruned analogue of adenophostin A.

The chosen disconnection of these base modified analogues indicates a common disaccharide intermediate (**124**) to which each base may be added see Figure **5.1**. This was found to be the most logical approach since regioselective protection of each nucleoside followed by glycosylation with the glucosyl donor would be more complex.

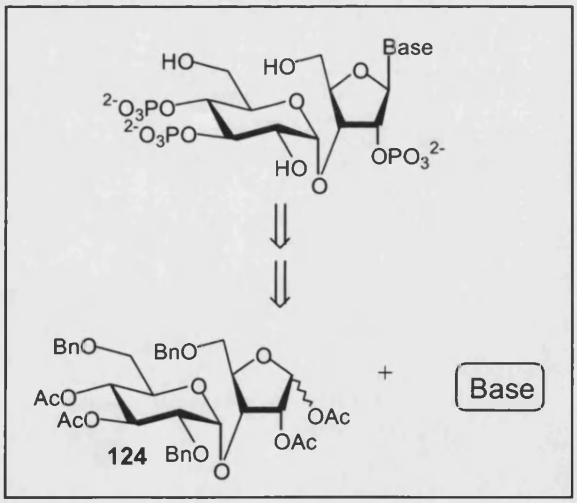


Figure **5.1**: Retrosynthetic basis for the synthesis of base modified analogues indicating the required disaccharide precursor

A recent synthesis of adenophostin A[160] adopted this disaccharide approach and detailed the synthesis of such an intermediate. Although this is the highest yielding

synthesis reported in the literature so far for adenophostin A, some of the disaccharide protecting group strategy involved more than one protection and deprotection for several functional groups. Since this was not ideal another protecting group strategy was sought. After a search of the literature a synthesis for the two target base modified adenophostin A analogues from a versatile disaccharide intermediate was designed with a single protection and deprotection step for all but the ribose 1,2-diol. Benzyl ethers were chosen to mask those positions that needed to be protected during phosphorylation, and the more labile acetate group was chosen to protect positions where phosphorylation was required. Acetate protection was also crucial to the Vorbrüggen condensation, since it is the presence of acyl groups at the 1- and 2-posittions of the disaccharide which controls the regioselectivity of the condensation. Thus the glycosyl donor and acceptor were prepared from known intermediates with this protecting group strategy in mind, and then coupled together using phosphite glycosidation methodology give a disaccharide intermediate.

5.2 Synthesis of a versatile disaccharide intermediate

5.2.1 Synthesis of the acceptor

The desired acceptor was easily prepared from commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose (**125**).[199] Selective alkylation of the 5-hydroxyl was achieved by two different methods. The first method was investigated by a colleague (D. J. Jenkins), and involved the regioselective ring opening of a 3,5-*O*-dibutylstannylene derivative with benzyl bromide to yield the 5-*O*-benzyl ether in one step. A 78% yield of benzylated products was isolated containing 90% of the desired 5-*O*-benzyl regioisomer (**128**) as indicated by ^{1}H NMR. This method has since been reported independently by another group.[200]

The other method involved the selective ring opening of a 3,5-*O*-benzylidene acetal. Treatment of 1,2-*O*-isopropylidene-α-D-xylofuranose with benzaldehyde dimethyl acetal in the presence of catalytic *p*TSA at 70°C with continuous removal of liberated methanol gave the desired acetal (**126**) as a single diastereoisomer in 89% yield, with the ^{1}H NMR spectrum showing a singlet at δ_H 5.45 corresponding to the benzylidene C*H*. Regiospecific cleavage of this acetal was achieved in 68% yield with a

mixture of 1.0M sodium cyanoborohydride in THF and 1.0 M hydrochloric acid in ether to yield the desired 5-*O*-benzyl ether. These reagents were chosen since they are known to cleave benzylidene acetals at the most hindered oxygen,[201] the selectivity being due to the small electrophilic proton favouring protonation at O-3 rather than O-5. The cleavage product structure was verified by acetylation. ^{1}H NMR of the acetate derivative indicated a deshielded signal at δ_H 5.25, corresponding to H-3. The combined yield for this two step alkylation of the 5-hydroxyl however was 60%, thus the higher yielding single step method was preferred for the preparation of this intermediate.

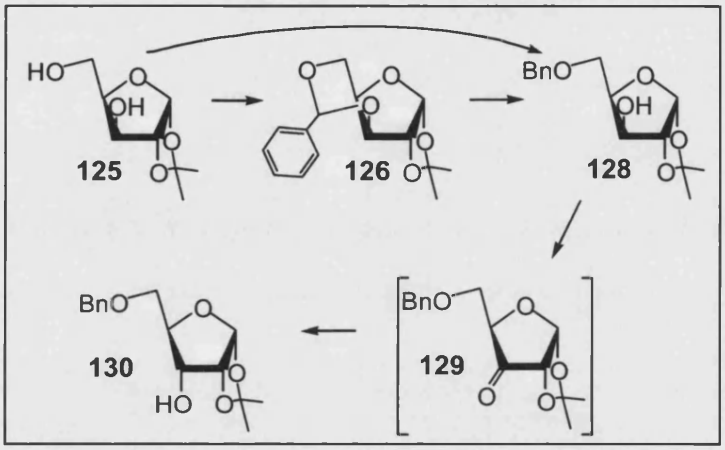


Figure **5.2**: Synthesis of glycosyl acceptor **130**.

Inversion of the 3-hydroxyl to give the ribofuranoside acceptor (**130**) was achieved by oxidation of this position with acetic anhydride and DMSO and reduction of the 3-ulose intermediate with $NaBH_4$[200](and references therein).

5.2.2 Synthesis of the donor

The free 3 and 4 positions of **65** meant it was an ideal starting material for the synthesis of the glycosyl donor. Treatment of **65** with acetic anhydride in pyridine gave the diacetate in quantitative yield. The acetylated positions gave rise to two deshielded triplets with large diaxial coupling constants (J 9.8 Hz), corresponding to H-3 and H-4 in the ^{1}H NMR spectrum.

The conditions used for the cleavage of the allyl glycoside of **66** were not suitable for the analogous reaction with **131** since treatment with the potassium *t*-butoxide would have cleaved the acetate protecting groups. A milder method was however found involving $PdCl_2$.[198] Under these conditions **131** was smoothly converted into the

regioselectively protected glucopyranose **132**. Finally phosphitylation in the manner described in chapter **2** gave the phosphite donor **133** in a 1:1 anomeric mixture as judged by ^{1}H and ^{31}P NMR.

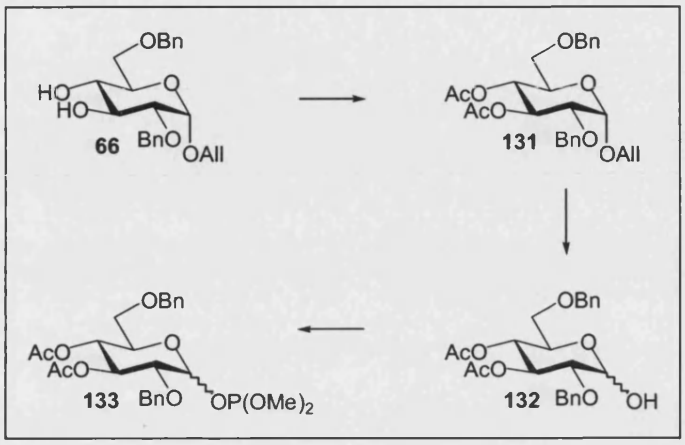


Figure **5.3**: Route to glycosyl donor **133**.

5.2.3 Disaccharide synthesis

Donor **133** and acceptor **130** were coupled together in a similar fashion to that described in the synthesis of furanophostin to give the desired disaccharide in 81% yield. In this case however, the reaction time was extended because of the deactivating effects of the donor 3,4-di-*O*-acetate protecting groups which are known to decrease the reactivity of a donor.[195]

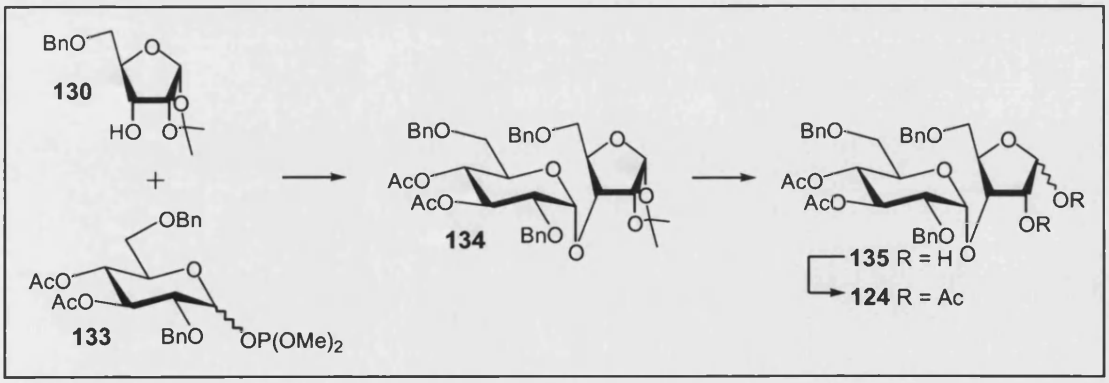


Figure **5.4**: Route to versatile disaccharide intermediate **124**.

Of note is the high regiospecificity of this glycosylation, the α-coupled anomer being the sole isolated product in high yield. The α-configuration about the new glycosidic bond was easily identifiable from the ^{1}H NMR spectrum, which presented a

deshielded doublet at δ_H 5.20 with a characteristically small coupling constant of J 3.9 Hz corresponding to H-1'.

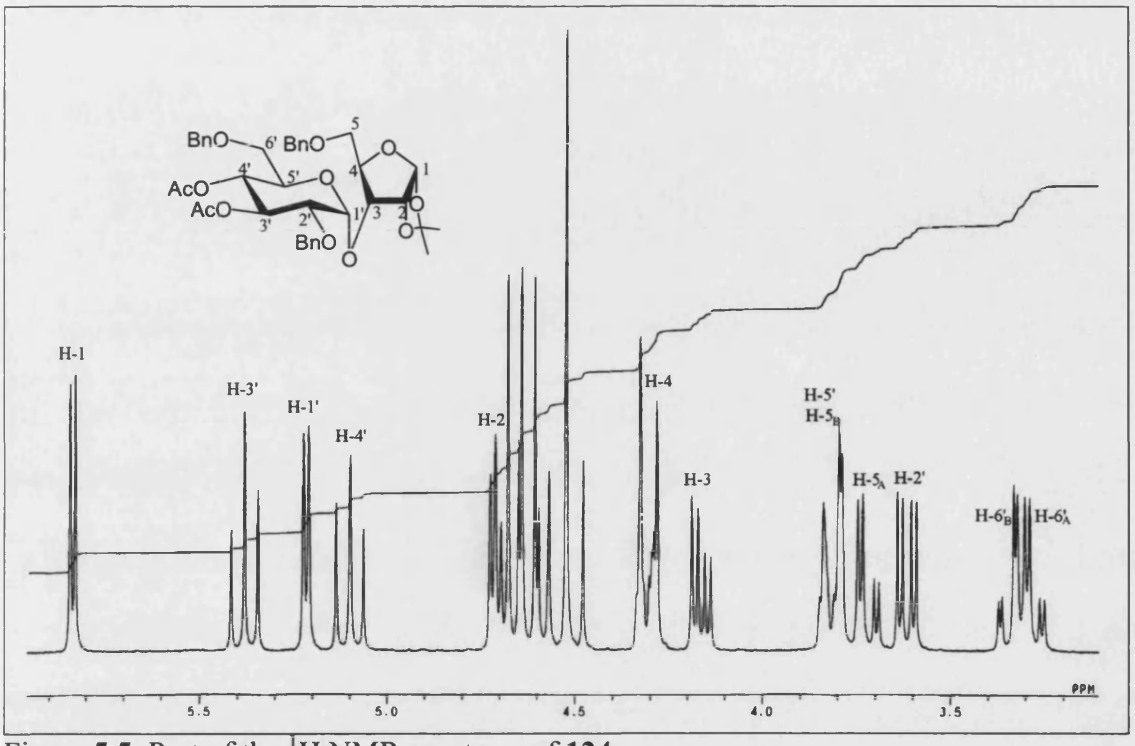


Figure **5.5**: Part of the ^{1}H NMR spectrum of **124**.

Cleavage of the isopropylidene acetal was accomplished by heating **134** at reflux for a short time in a mixture of acetic acid and water containing ethylene glycol.[160] The 1,2-di-*O*-acetate (**124**) was then prepared by treatment with acetic anhydride, in order to meet the Vorbrüggen condensation criteria of acyl groups at these positions.

5.3 Synthesis of purinophostin

The first base modified analogue synthesized involved the effective deletion of N^6 to leave the purine base. It was thought that this amine group may enhance the interaction of the adenophostins with the Ins(1,4,5)P_3 receptor by acting as a hydrogen bond donor, and that its absence may result in a drop in activity.

5.3.1 Hilbert Johnson-Vorbrüggen condensation and deprotection

Synthesis of nucleosides *via* the reaction of silylated heterocyclic bases with peracetylated sugars in the presence of Friedel-Crafts catalysts is now well established. The use of TMSOTf as the catalyst was first described by Vorbrüggen *et al.* in 1981.[202] Previously $SnCl_4$ had been used, but it was difficult to remove in the work up. TMSOTf was found to be a better catalyst because it was easily removed in the work up and gave higher yields.

In a subsequent paper Vorbrüggen and Höfle[203] reported an investigation into the mechanism of nucleoside formation with TMSOTf as catalyst. They found that TMSOTf converted the 1,2-di-*O*-acyl ribofuranoside into an electrophilic 1,2-acyloxonium salt and it also formed a σ-complex with the silylated base. These two intermediates then reacted together to form the nucleoside. With this discussion in mind the prerequisite for the 1,2-di-*O*-acteate of disaccharide **124** becomes apparent.

Before the condensation of **124** with purine was attempted a model reaction was carried out to confirm that the nucleosidic bond would be formed at the desired purine N^9-position. It is known that treatment of 2',3',5'-tri-*O*-acetyl adenosine with *n*-pentyl nitrite gives 2',3',5'-tri-*O*-acetyl 9-β-D-ribofuranosidopurine (**142**) for which full characterization data have been published.[204] It was reasoned that condensation of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose and silylated purine in the presence of TMSOTf should give a product identical to **142**. The isolated product from this reaction did indeed give ^1H and ^{13}C NMR spectra in agreement with those from 2',3',5'-tri-*O*-acetyl adenosine treated with *n*-pentyl nitrite. In addition the fully deprotected product from the Vorbrüggen condensation had spectra in agreement with those for 9-β-D-ribofuranosidopurine (nebularine).

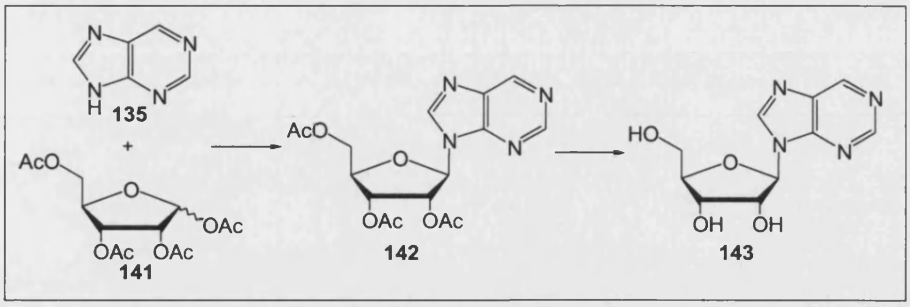


Figure **5.6**: Synthesis of nebularine (**143**).

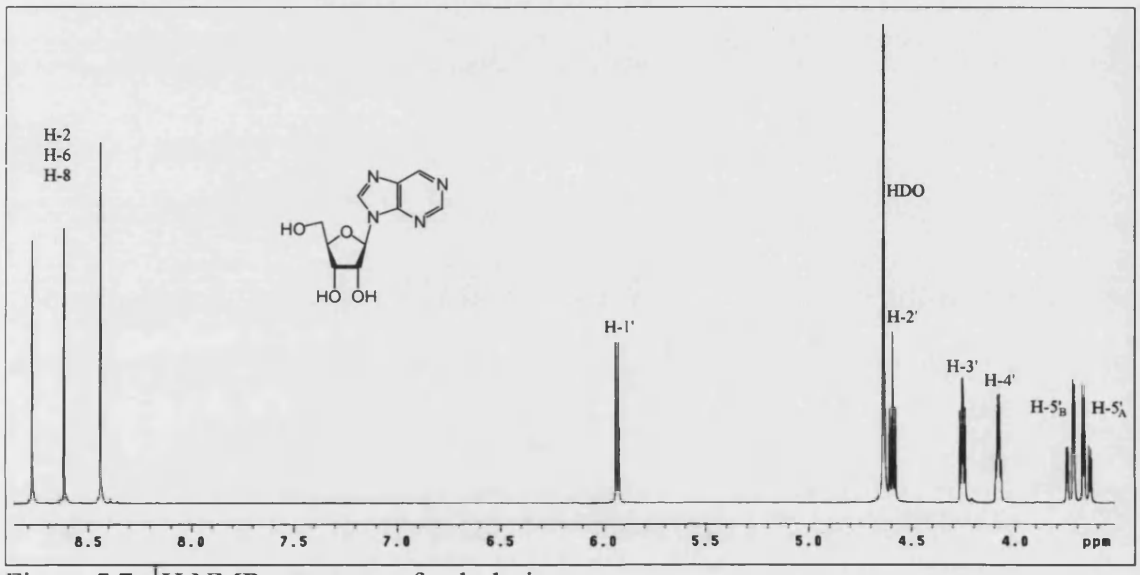


Figure **5.7**: ^{1}H NMR spectrum of nebularine.

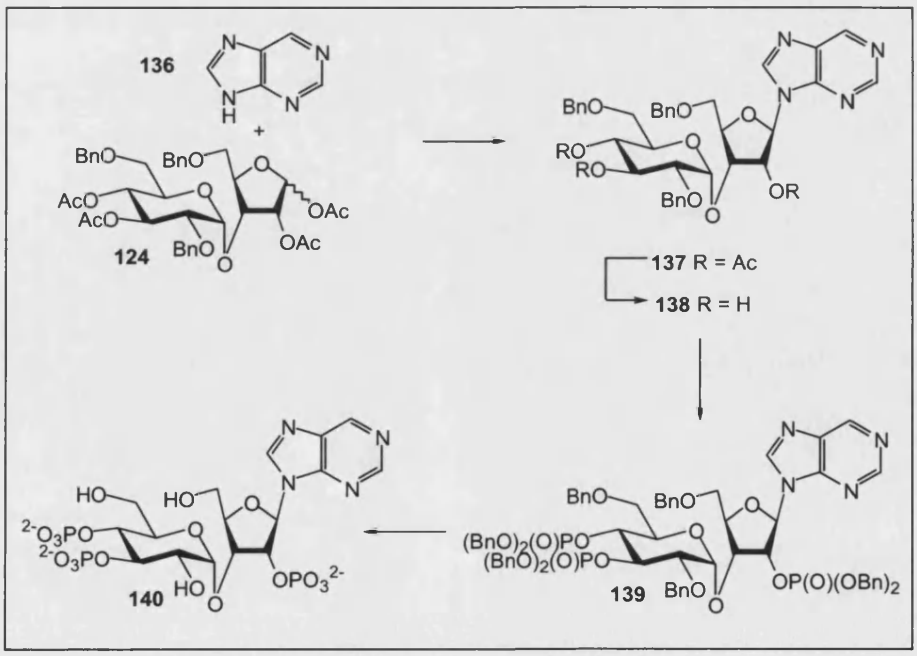


Figure **5.8**: Route to purinophostin (**140**).

After the success of the model Vorbrüggen condensation reaction the condensation of purine with disaccharide **124** was attempted. TLC during the course of the reaction saw the formation of two products. Initially the lower spot was the major one, but over time the higher spot began to predominate. Extended reaction times did not however see complete conversion to the upper spot. The reaction was carried out successfully with both catalytic and stoichiometric amounts of TMSOTf with no significant difference in proportions of the major and minor products. Catalytic TMSOTf was slower though, taking 24 hours for complete reaction of starting material and equilibration of products.

The major product was identified as the 9-β-D-ribofuranosidopurine nucleoside derivative (**137**). The characteristic pattern of purine ring-carbon signals found in the ^{13}C NMR of 2',3',5'-tri-*O*-acetyl 9-β-D-ribofuranosidopurine was clearly exhibited by the ^{13}C NMR spectrum of **137**, while the ^{1}H NMR of **137** exhibited a deshielded doublet at δ_H 6.30 corresponding to H-1'. Unfortunately the identification of the regioisomer proved more difficult and with the short time span available no definitive assignment was made.

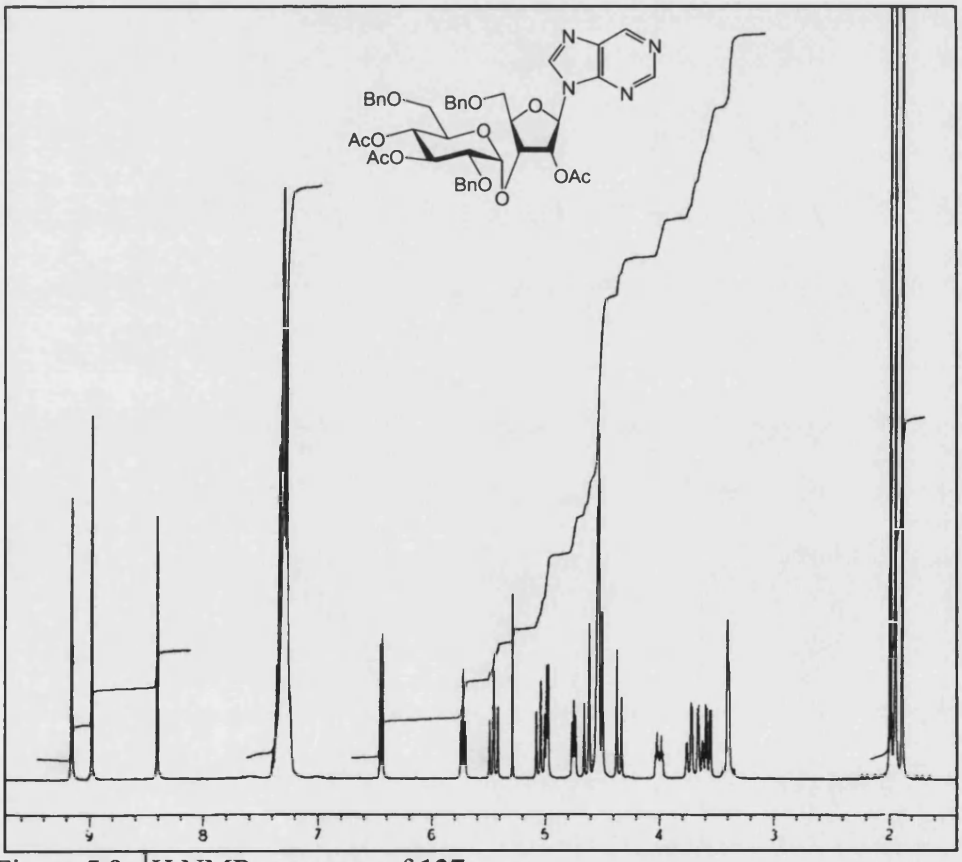


Figure **5.9**: ^{1}H NMR spectrum of **137**.

Cleavage of the acetate esters was straightforward. Fully protected intermediate **137** was stirred in a mixture of concentrated aqueous ammonia and methanol in a sealed flask overnight to give the triol **138** required for phosphorylation.

5.3.2 Phosphorylation and deprotection

Triol **138** was phosphitylated with a pre-formed complex between tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in DCM. The resulting trisphosphite was oxidised with MCPBA to the trisphosphate (**139**). The ^{1}H-coupled ^{31}P NMR spectrum of the purified product exhibited three overlapping sextets corresponding to the three dibenzyloxy phosphate groups.

Deprotection of **139** to give the purine analogue of adenophostin A was achieved with catalytic transfer hydrogenation in a similar manner to the deprotection of the adenophostin A precursor. Complete deprotection did however take 17 hours compared to the 2 hours needed to deprotect the corresponding adenophostin A precursor, and this probably lowered the yield. The crude product was purified on MP1 AG ion exchange resin, and eluted with a 0–100% gradient of 150mM aqueous TFA. Great care was required during evaporation of the eluent as depurination was found to occur if the water bath temperature was not kept below 20°C. ^{1}H NMR of the clear glassy product indicated complete loss of all the benzyl protecting groups, and ^{31}P NMR exhibited three resonances from the three deprotected phosphates. The isolated free acid was finally converted into the sodium salt by eluting through a short column of sodium exchange resin. Quantification was carried out with the Briggs phosphate assay and by UV, both of which were in agreement. A sample of purinophostin was examined by HPLC and the resulting HPLC trace is shown below in Figure **5.10**.

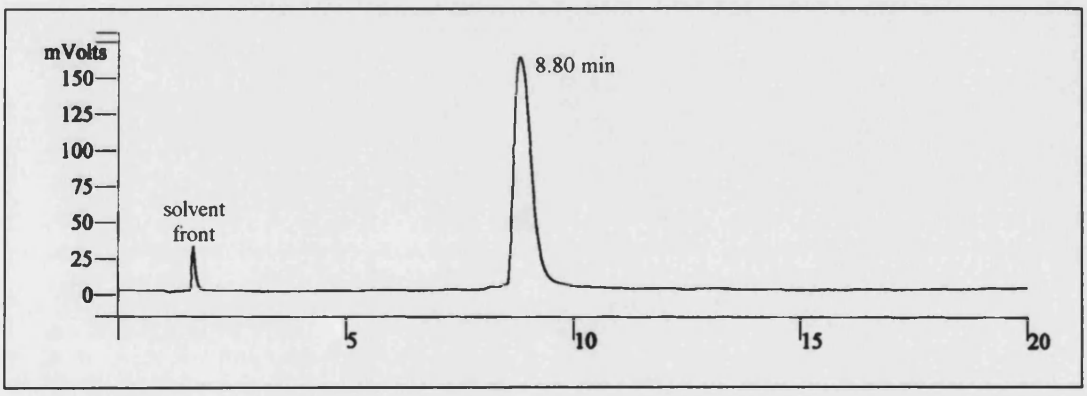


Figure **5.10**: HPLC trace of purinophostin.

5.4 Biological results

Biological testing of purinophostin for Ca^{2+} mobilisation in permeabilised hepatocytes was carried out by a collaborator using the method described in chapter **2**. The results are shown in Table **5.1**. Purinophostin was found to be essentially equipotent with adenophostin A.

	EC_{50} (nM)	*h*	Maximal response %	n
Purinophostin	18±1	2.04±0.24	52±3	10
Ins(1,4,5)P_3	119±8	2.69±0.55	55±3	15
Adenophostin A	14±2	1.99±0.18	51±6	6

Table **5.1**. $^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3, adenophostin A and purinophostin. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[180] Results are shown as means ± S.E.M.

5.5 Synthesis of imidophostin

The second base modified analogue constituted the deletion of the adenine six membered ring to leave the imidazole moiety. It is known that the position of the base at the nucleosidic bond can adopt two main orientations relative to the sugar moiety, called *syn* and *anti* (Figure **5.11**).

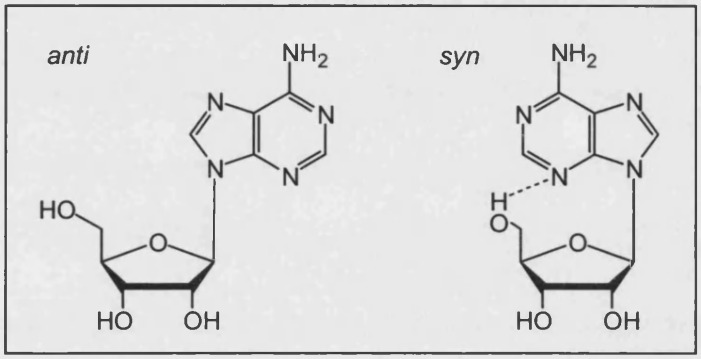


Figure **5.11**: *Syn* and *anti* conformations of adenosine.

Often the *syn* conformation in adenosine is stabilized by a $O^{5'}$–N^3 intramolecular hydrogen bond. In an imidazole analogue there is no opportunity for such an interaction to take place and so the conformation about its nucleosidic bond may be completely different to that of the adenophostins. These considerations may also have some bearing on the ring puckering of the ribose ring and thus the positioning of the 2'-phosphate group. In addition N^1 is often a hydrogen bond acceptor; such an interaction with the Ins(1,4,5)P_3 receptor may contribute to the adenophostins enhanced binding affinity. The absence of N^1, and indeed the pyrimidine ring in the imidazole analogue may affect binding to the receptor.

5.5.1 Hilbert Johnson-Vorbrüggen condensation and deprotection

To the author's knowledge there is only one reported synthesis of *N*-(β-D-ribofuranosyl)imidazole in the literature,[205] although other groups must have prepared it because they have described its crystal structure,[206] circular dichroism[207] and molecular orbitals.[208] The method employed to prepare *N*-(β-D-ribofuranosyl)imidazole is similar to that used in the synthesis of purinophostin (the silyl Hilbert Johnson-Vorbrüggen method), and involves TMSOTf catalysed condensation of *N*-trimethylsilylimidazole and

1,2,3,5-tetra-*O*-acetyl-D-ribofuranose. The formation of *N*-(*β*-D-ribofuranosyl) imidazole was also accompanied by the formation of the C_2 symmetrical bis-glycosylated compound in significant amounts, although a reduction in the amount of imidazole reagent used minimalised this.

The successful preparation of *N*-(*β*-D-ribofuranosyl)imidazole by this method meant that the preparation of an imidazole analogue of adenophostin A was easily accessible by condensation of disaccharide **124** already in hand with *N*-trimethylsilylimidazole (**144**).

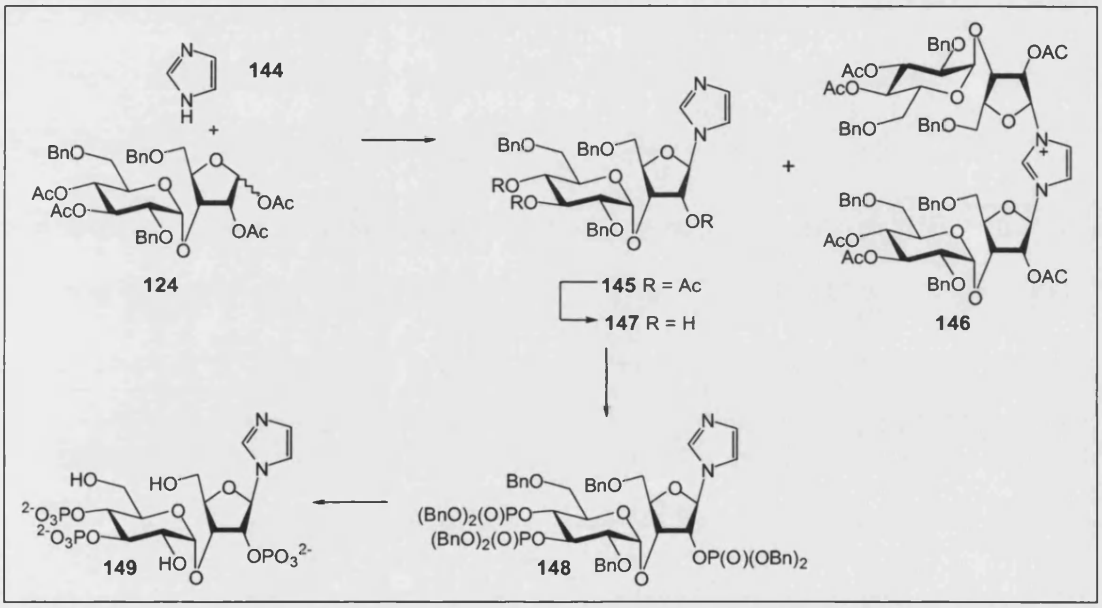


Figure **5.12**: Route to imidophostin (**149**).

Thus **124** and *N*-trimethylsilylimidazole were heated at reflux in 1,2-dichloroethane with TMSOTf for a total of 24 hours. The mono-glycosylated compound was the major product (**145**)(57%) and the bis-glycosylated compound the minor (20%) (**146**). Efforts to reduce the formation of bis-glycosylated compound met with little success. A reduction in the equivalents of *N*-trimethylsilylimidazole used lead to incomplete reaction of the starting material with bis-glycosylated compound still being formed.

Identification of the major product as the mono-glycosylated compound from the ^{1}H NMR spectrum was based on the loss of symmetry in the imidazole ring, and the integral of each imidazole ring proton signal being one. H-4 and H-5 were distinctly separate signals at δ_H 7.14 and 7.08 respectively while H-2 resonated at δ_H 7.82. The ^{13}C

NMR spectrum reiterated this pattern with a marked difference between the signal shifts for C-4 and C-5 (δ_C 116.84, C-5, and δ_C 129.52, C-4). In addition the peak at *m/z* 759 of the positive ion FAB mass spectrum corresponded to $(M+H)^+$ of the mono-glycosylated product.

The structure of the bis-glycosylated product was identified in a similar way. Both the ^1H and ^{13}C NMR spectra exhibited only two signals for the imidazole ring protons. The ^1H NMR was especially distinctive with one broad singlet for H-4 and H-5 at δ_H 7.51 integrating for two protons and a highly deshielded broad singlet at δ_H 9.37 for H-2 integrating for one proton. Furthermore the rest of the ^1H NMR spectrum signals all integrated for twice the number of protons seen in the mono-glycosylated ^1H NMR spectrum. Finally the positive ion FAB mass spectrum for this compound exhibited a peak at *m/z* 1449 corresponding to the bis-glycosylated, positively charged imidazolium ion.

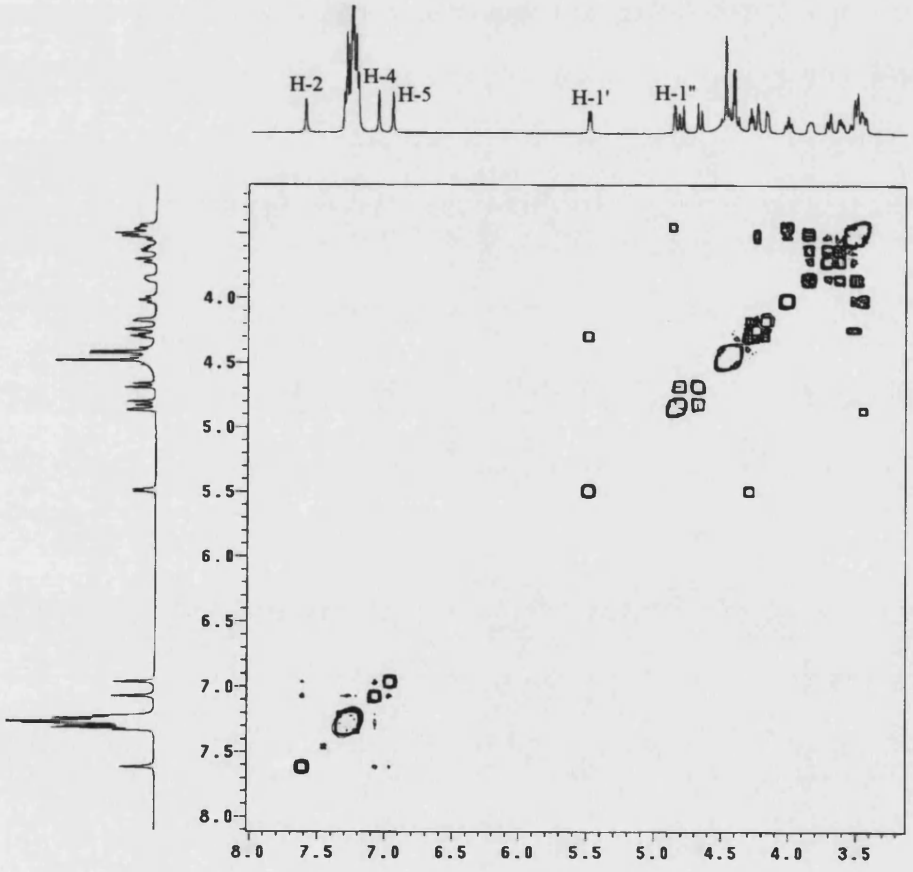


Figure **5.13**: Part of the ^1H-^1H COSY NMR spectrum of the triol **147**.

As with the corresponding purinophostin intermediate, cleavage of the acetate esters of **145** was achieved by stirring in a mixture of concentrated aqueous ammonia and methanol in a sealed flask overnight. Purification of the product gave the desired triol **147** ready for phosphorylation.

5.5.2 Phosphorylation and deprotection

Phosphitylation of **147** with tetrazole and bis(benzyloxy)(diisopropylamino) phosphine in DCM went smoothly to give the trisphosphite which was oxidised to the trisphosphate with MCPBA. The ^{1}H-coupled ^{31}P NMR spectrum exhibited three characteristic sextets corresponding to the three protected phosphate groups.

Again complete deprotection was achieved with catalytic transfer hydrogenation. **148** Was heated at reflux in a mixture of water, methanol and cyclohexene with 20% palladium hydroxide on carbon for 10 hours. After removal of the catalyst by filtration the crude product was purified by ion exchange chromatography with a gradient of 0–100% 150 mM aqueous TFA. The fractions containing product (**149**) were identified by a qualitative Briggs test and those found to contain phosphate were concentrated to a clear glassy solid. ^{1}H NMR spectroscopy indicated complete loss of all the benzyl protecting groups and as expected no imidazole ring reduction. This was in agreement with the ^{1}H-coupled ^{31}P NMR spectrum, which exhibited three doublets. Quantification was only carried out by the Briggs phosphate assay since this analogue had little UV activity. The isolated free acid was then finally converted into its sodium salt by eluting though a sodium ion exchange column.

5.6 Biological results

	EC_{50} (nM)	h	Maximal response %	n
Imidophostin	108±20	2.51±0.64	57±3	7
$Ins(1,4,5)P_3$	119±8	2.69±0.55	55±3	15
Adenophostin A	14±2	1.99±0.18	51±6	6

Table **5.2**. $^{45}Ca^{2+}$ release data for $Ins(1,4,5)P_3$, adenophostin A and imidophostin. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[180] Results are shown as means ± S.E.M.

Imidophostin was biologically tested for Ca^{2+} mobilisation in permeabilised hepatocytes by a collaborator according to the method described for adenophostin A in chapter **2**. The results shown in Table **5.2** indicate that imidophostin was only marginally more active than $Ins(1,4,5)P_3$.

5.7 Further base modified analogues

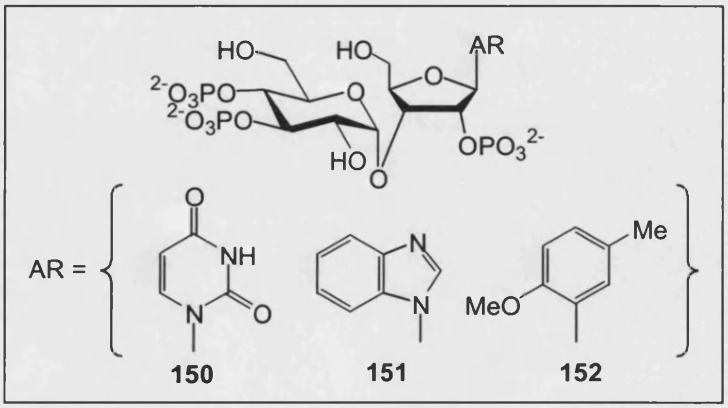


Figure **5.14**: Further base modified analogues

The disaccharide **124** was considered an ideal intermediate for the synthesis of base modifies analogue of adenophostin A, and as such it was used in our laboratory by

Professor S. Shuto in the synthesis of **150–152**. These analogues were designed to investigate the effect of replacing adenine with other aromatic base substitutes, with a view to elucidating the key properties of adenine which contribute to the outstanding activity of adenophostin A.

Substitution of adenine for uracil was achieved using standard Vorbrüggen methodology, and the resulting adenophostin A analogue was named uridophostin. When tested for Ca^{2+} release from permeabilised hepatocytes uridophostin exhibited an EC_{50} of 34 nM, while adenophostin A tested gave an EC_{50} of 9.6 nM recently. The 3-fold difference in the activities of adenophostin A and uridophostin would suggest that the type of interaction between the adenine of adenophostin A and the imidazole of imidophostin with residues of the Ins(1,4,5)P_3 receptor differs only slightly. It is clear from comparison of the structures of adenine and uracil that the positions available for hydrogen bonding are spatially quite different, and so it is plausible that the main interaction of this part of the molecule with the receptor relies on aromatic character rather than any hydrogen bonding, with hydrogen bonding playing a more accessory role. This conclusion is supported by the results from the benzimidazole and 4-methyl anisole analogues, which gave EC_{50} values of 47 nM and 48 nM respectively. Both of these analogues possess base substitutes which lack the ability to form hydrogen bonds in the same way as the N^1 and N^3-positions of adenine.

Furthermore it is feasible that the marginally lower potency of these analogues compared with adenophostin A may have arisen from differences in binding conformation at the receptor. As mentioned earlier, hydrogen bonding between N^3 of the adenine ring and the 5'-hydroxyl is possible in adenophostin A; in the above base modified analogues such an interaction is impossible.

Finally the condensation of the disaccharide intermediate **124** with 4-methylanisole gave some α-product. This material was used to make the α-4-methyl anisole analogue of adenophostin A. The ability of this analogue to mobilise Ca^{2+} was almost 30-fold lower (EC_{50} 1372nM) than the analogous β-linked analogue. Thus it is clear that the conformation about the ribofuranoside anomeric position is extremely important to the activity of adenophostin A.

5.8 Summary

The similar Ca^{2+} mobilising properties of purinophostin and adenophostin A suggest that the N^6 amine of adenophostin A does not play a significant part in its binding at the $Ins(1,4,5)P_3$ receptor. However the weaker activity of imidophostin indicates clearly that the imidazole ring is not enough for adenophostin A-like activity. Perhaps the imidazole of imidophostin is not large enough to reach fully the binding pocket occupied by the adenine of adenophostin A. Or it may be that the imidazole moiety is not be able to engender the same hydrophobic interactions with the receptor that are possible with the adenine of adenophostin A. In addition the absence of N^3 of adenine in imidophostin means that there is no hydrogen bond acceptor to stabilise its conformation through hydrogen bonding with the 5'-hydroxyl. Although weaker than adenophostin A, imidophostin is however somewhat more active than both ribophostin and furanophostin, this suggests that the either the imidazole moiety alters the conformation of the molecule resulting in a more optimal binding conformation, or it directly enhances binding at the $Ins(1,4,5)P_3$ receptor to give increased activity.

It is also possible that the imidazole moiety was protonated at the pH at which the biological evaluation took place, since imidazole has a pKa of 6.92. Hence the activity of imidophostin needs to be examined at a range of pH values to investigate the effect of its ionisation on its ability to release Ca^{2+}.

Thus the biological results of purinophostin, imidophostin and the analogues synthesised by Professor S. Shuto suggest that the interaction of the adenine base of adenophostin A with the $Ins(1,4,5)P_3$ receptor is probably a hydrophobic one, and that hydrogen bonding and possibly a conformational effect from the analogue base may play a smaller role.

Chapter six

Recent background and general conclusions

6 Recent background and general conclusions

6.1 Recent background

6.1.1 Further adenophostin A analogues modified at either the ribose or glucose moieties

After this work was commenced the synthesis of adenophostin A and several other adenophostin A analogues were published by van Straten *et al.*[160;209;210] In addition the biological properties of some of these analogues (**153–157**) were reported recently.[211] These adenophostin A analogues shown in Figure **6.1** were modified at the ribose and glucose portions of the molecule.

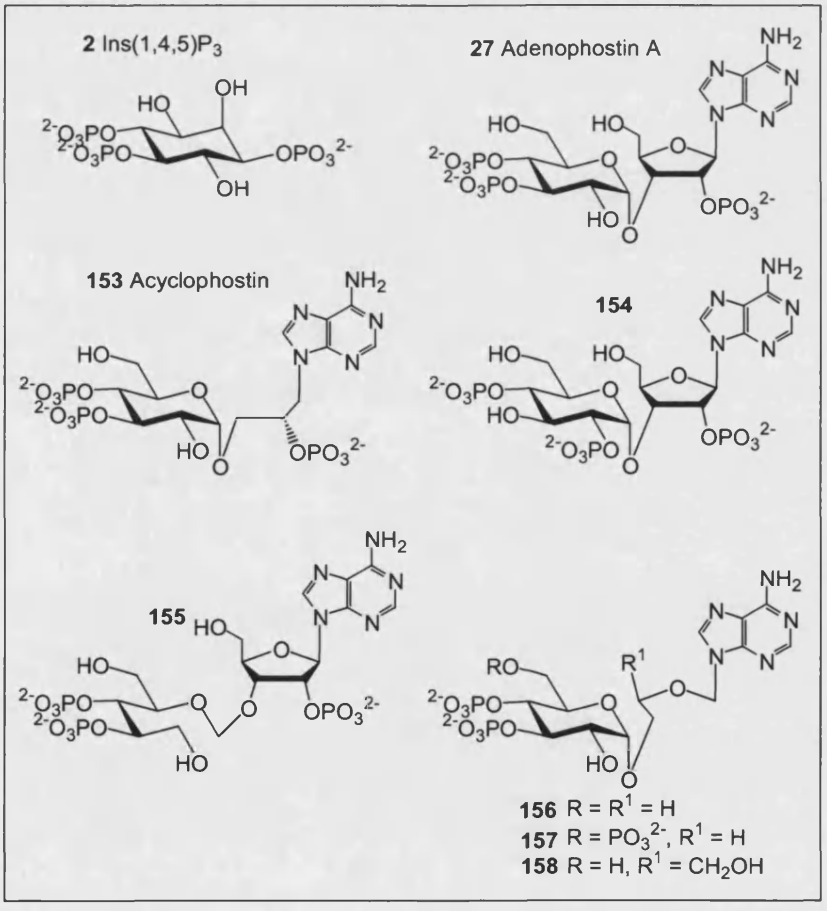


Figure **6.1**: Adenophostin A analogues modified at either the ribose or glucose moieties.

The ability of these analogues to release Ca^{2+} from the intracellular stores of permeabilised hepatocytes was investigated. Analogues **155** and **157** were found to be inactive at concentrations of >100 μM. Their inactivity however, was to be expected, since neither possessed the structural motif assumed to be necessary for activity at the Ins(1,4,5)P_3 receptor. Namely, a vicinal bisphosphate with the same relative stereochemistry as Ins(1,4,5)P_3, a neighbouring hydroxyl corresponding to the 6-hydroxyl of Ins(1,4,5)P_3 and a third suitably positioned phosphate. In particular, the disruption of the glucose-related ring in analogue **155** probably allowed too much conformational mobility around the bisphosphate and neighbouring hydroxyl. While the position of the third phosphate of analogue **157** is clearly not analogous to that of adenophostin A or Ins(1,4,5)P_3.

The third phosphate at the 2'-position of adenophostin A has been demonstrated to be crucial to its binding at the Ins(1,4,5)P_3 receptor, since its deletion resulted in a ~2000-fold reduction in affinity for the receptor[159] relative to adenophostin A. This loss in activity was paralleled by the activity of analogue **156**, which exhibited >3000-fold decrease in affinity for the Ins(1,4,5)P_3 receptor compared with adenophostin A. Thus it may be concluded that the mobility of analogue **156**, resulting from the loss of C-2' of the ribose ring, in conjunction with the loss of the 2'-phosphate further decreases the ability of **156** to bind to the Ins(1,4,5)P_3 receptor.

Analogue **154** was found to be 100-fold weaker than Ins(1,4,5)P_3 in both Ca^{2+} release and binding to the receptor. This was surprising since it does not possess a vicinal bisphosphate. The most probable explanation for the activity of this analogue was that a protecting group migrated during a prephosphorylation stage of its synthesis. <1% Contamination of **154** with adenophostin would account for its observed activity.

The analogue named acyclophostin exhibited unusual activity. It bound to hepatic membranes with appreciably higher affinity than Ins(1,4,5)P_3, yet it was marginally less potent in causing Ca^{2+} mobilisation. All the other analogues investigated along with acyclophostin had similar EC_{50}/K_d ratios, whereas the ratio for acyclophostin was significantly higher. Such characteristics led to the suggestion that acyclophostin was a partial agonist. Further investigation demonstrated that acyclophostin was in fact a pH dependent partial agonist, it was found to be a partial agonist at high pH (8.3) and a full agonist at pH 7. It was speculated that the pH dependent efficacy of acyclophostin was either the result of a conformational change related to the pKa of the 2'-phosphate

and the lack of conformational restriction from a furanoside ring, or that acyclophostin may differ from Ins(1,4,5)P_3 in the residues involved in its binding and Ca^{2+} release at the Ins(1,4,5)P_3 receptor.

6.1.2 An investigation into the solution conformation of adenophostin A

Using a combination of molecular mechanics calculations and NMR Hotoda *et al.*[212] have suggested a putative conformation for adenophostin A at the Ins(1,4,5)P_3 receptor binding site. Their suggested conformation accounts for NOE data observed between H-8 and H-1', and between H-8 and H-2'. They propose that their model indicates the possibility that the relative position of the 2'-phosphate group of adenophostin A is optimal for the recognition by the Ins(1,4,5)P_3 receptor. They found that the ribose ring of adenophostin A prefers the *C*2'-*endo* form, while the ribose ring of ribophostin gave the *C*3'-*endo* form. The *C*2'-*endo* form results in a more extended conformation for adenophostin A, and a greater distance between the bisphosphate and the single phosphate on the ribose ring compared with ribophostin. Furthermore the N-glycosyl linkage was found to be in the *syn* orientation.

These findings are consistent with the results of the work in this thesis; i.e. that the adenine of adenophostin A induces a conformation in the molecule that results in optimal binding at the Ins(1,4,5)P_3 receptor, and/or the adenine moiety directly interacts with the Ins(1,4,5)P_3 receptor in close vicinity to the Ins(1,4,5)P_3 binding pocket.

6.2 Summary of the work in this thesis

6.2.1 Synthetic work

An efficient route to the synthesis of adenophostin A has been devised and completed. The regioselective protection of adenosine to give 5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine (**40**) included a new selective dimethoxytritylation of the adenine N^6 position and a key regioselective reduction of a *p*-methoxybenzylidene acetal. Glycosidation of **40** with a fully protected glucosyl phosphite followed by cleavage of all the acid sensitive groups gave the desired triol ready for phosphitylation. Phosphitylation was achieved in the presence of a free N^6-position by a recently reported method involving imidazolium triflate. The resulting trisphosphite was oxidised to the trisphosphate with MCPBA and final debenzylation was accomplished by catalytic hydrogenation to give adenophostin A. The synthesis of two sugar modified analogues, mannophostin and xylophostin, was achieved using the preceding strategy and glycosidation with either a mannose or xylose phosphite.

A concise route to the minimal structure analogue ribophostin, was devised and completed using trichloroacetimidate glycosidation methodology. While the synthesis of another minimal structure analogue was achieved using phosphite glycosidation methodology.

An efficient route to the synthesis of a versatile disaccharide intermediate (**124**) from a regioselectively protected ribofuranose glycosyl acceptor and a glucosyl phosphite donor and was completed. Vorbrüggen condensation of this intermediate with purine and imidazole led to the synthesis of two base modified analogues of adenophostin A, purinophostin and imidophostin.

6.2.2 Structure-activity relationship work

Along with the synthesis of adenophostin A, the syntheses of six adenophostin A analogues have been presented in this thesis. Their biological evaluation in direct comparison with our synthetic adenophostin A and Ins(1,4,5)P_3 has allowed several important conclusions to be drawn concerning the structure-activity relationships of adenophostin A at the Ins(1,4,5)P_3 receptor. These are summarised in Figure **6.2**.

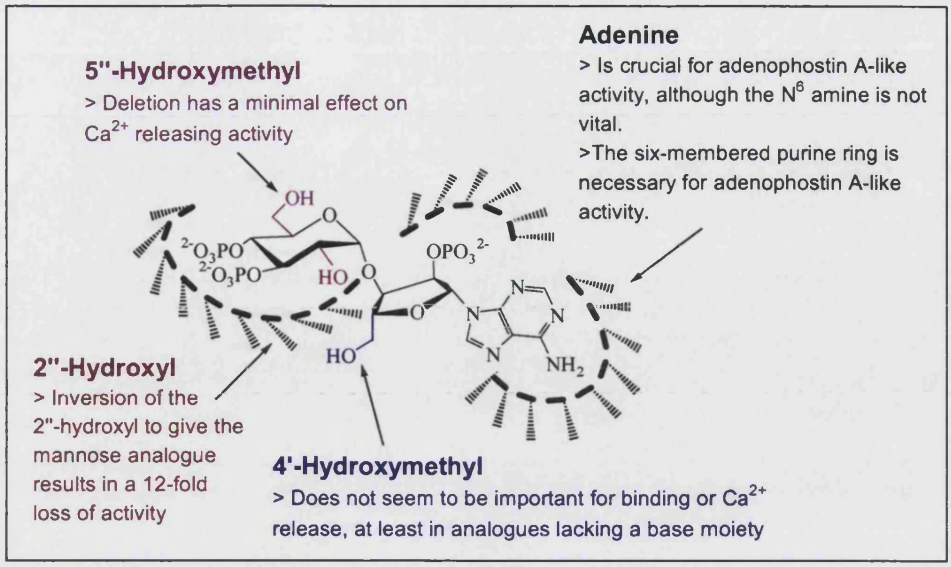


Figure **6.2**: The properties of some of the structural features of adenophostin A involved in activity at the Ins(1,4,5)P_3 receptor.

From the structure-activity information in Figure **6.2** it is possible to propose a binding model for adenophostin A at the Ins(1,4,5)P_3 receptor. The 3",4"-bisphosphate, adjacent equatorial 2"-hydroxyl, and well positioned accessory 2-phosphate of adenophostin A comprise the basic structural motif that elicits similar activity to that of Ins(1,4,5)P_3, and thus probably binds in a similar way to the analogous motif of Ins(1,4,5)P_3. But for activity to approach that of adenophostin A the adenine base (or a similar structure) is vital. The interaction of this adenine base with the Ins(1,4,5)P_3 receptor is most likely a hydrophobic one, and probably has two main functions, either to optimise the conformation of adenophostin A at the Ins(1,4,5)P_3 receptor, and/or a direct interaction with nearby residues of the Ins(1,4,5)P_3 receptor to improve binding.

6.3 Overall summary of the structure-activity relationships of adenophostin A at Ins(1,4,5)P_3 receptors

By taking into account the recent reports in the literature for adenophostin A analogues, and combining these with the conclusions from section **6.2** it was possible to further summarise some of the features of the structure of adenophostin A responsible for

its exceptional activity at the Ins(1,4,5)P_3 receptor. This summary is depicted in Figure **6.3**.

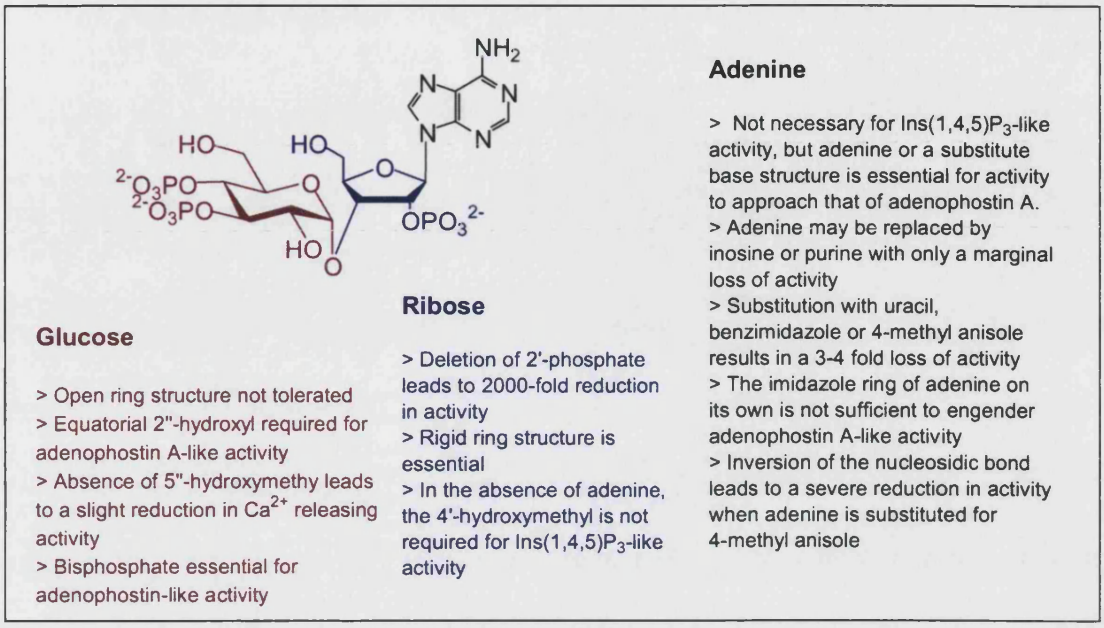


Figure **6.3**: Summary of the structure-activity relationships of adenophostin A at the Ins(1,4,5)P_3 receptor.

6.4 Future directions

We now have a better understanding of the structural features of adenophostin A that contribute to its exceptional activity, but an extension of this work in an effort to refine these ideas is desirable. Further investigation into the properties of the base moiety that are required for adenophostin A-like activity, using base modified analogues derived from the versatile disaccharide intermediate **124** would lead to a better understanding of the effect of the base moiety on binding interactions. In particular the investigation of conformational restriction about the nucleosidic bond would be interesting. Such an understanding, could then lead to a more informed approach to the design of analogues modified at the glucose portion of the adenophostin A structure, and may lead to the synthesis of more effective modulators of the Ins(1,4,5)P_3 receptor, possibly even antagonists.

Chapter seven

Experimental and References

7 Experimental and References

Experimental

7.1 General information

Thin layer chromatography (TLC) was performed on percolated plates (Merck TLC aluminium sheet silica 60 F_{254}Art no.5554); the compounds were detected by dipping in phosphomolybdic acid in ethanol followed by heating. Flash chromatography refers to the procedure developed by Still *et al.*,[213] and was carried out using Sorbsil C60 silica gel.

^{1}H and ^{13}C NMR were recorded on either JEOL GX270 or EX 400 or Varian Mercury 400 spectrometers. Chemical shifts were measured in part per million (ppm) relative to internal tetramethylsilane or D_2O. ^{31}P NMR spectra were also recorded on JEOL GX270 or 400 spectrometers and ^{31}P NMR NMR chemical shifts were measured in ppm relative to external 85% H_3PO_4. *J* values are given in Hz and the following abbreviation are used: s, singlet; d, doublet; dd, doublet of doublets*; ddd, doublet of doublet of doublets*; t, triplet; q, quartet; m, multiplet; br, broad; exch., exchanged with. Proton assignments were established with 2D COSY experiments, and the number of protons attached to carbon atoms was established by DEPT experiments.

Melting points were determined using a Reichert-Jung Therm Galen Kofler block and are uncorrected.

Microanalysis was carried out at the University of Bath Microanalysis Service.

Low resolution mass spectra were recorded at the University of Bath Mass Spectrometry Service using +ve and –ve fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol as the matrix. High resolution accurate mass spectra were recorded at the University of Bath Mass Spectrometry Service.

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* Where a dd or a ddd had a single coupling constant value, this value is reported only once in the ^{1}H NMR assignments.

Optical rotations were measured at ambient temperature using an Optical Activity Ltd AA-10 polarimeter in a cell volume of $1cm^3$ or $5cm^3$ and specific rotation are given in 10^{-1} deg cm^3 g^{-1}. Ion exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using either Sepharose Q fast flow resin and gradients of triethylammonium bicarbonate as eluent, or MP1 AG ion exchange resin and a gradient of 150mM TFA as eluent.

HPLC analysis was carried out on a Dynamax model SD-200 with reverse phase column: APEX ODS II 5μ S/N 7121103. A gradient of 0.05M phosphate buffer containing 0.1% tetrabutylammoniumhydrogen sulphate, and acetonitrile was used as eluent at 1.5mL/min, with a UV detector set at 259 nm.

Synthetic phosphates were assayed by adaptations of the Briggs phosphate test[179] as follows. For the qualitative test, $200mm^3$ aliquots of the ion exchange column fractions were pipetted into a test tube and the solutions were evaporated to dryness at 200°C. For drops of concentrated sulphuric acid were added to each tube, which was then heated in an oven at 200°C for 90min. The tubes were cooled to room temperature (rt), and water ($250mm^3$) was added with shaking to dissolve. To each tube were added $500mm^3$ of a solution of ammonium molybdate (2.5g) in water (20 cm^3) and concentrated sulphuric acid (8 cm^3), followed by $250mm^3$ of a solution of quinol (100mg) in water ($20cm^3$) and one drop of concentrated sulphuric acid, then finally $250cm^3$ of a solution of sodium sulphite (4g) in water ($20cm^3$). Each tube was heated at the boiling point (bp) of the mixture for 10 s. A blue colour indicated the presence of inorganic phosphate.

For the quantitative assay the mixtures treated as above were transferred to volumetric flasks and made up to $10cm^3$ with water. The UV absorbances at 340nm were recorded using $3cm^3$ quartz cells. Concentration was calculated from a standard curve compiled from UV absorbance values of known concentrations of KH_2PO_4 treated as above and measured at 340nm.

Chemicals were purchased from Aldrich, Fluka, Lancaster and Sigma chemical companies.

Acetonitrile and DCM were distilled over calcium hydride and stored over 4Å molecular sieves. *N,N*-dimethylformamide (DMF) was dried over barium oxide, distilled under reduced pressure and stored over 4Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled and then stored over potassium hydroxide pellets.

Tetrahydrofuran (THF) was dried by passing through activated alumina to expel peroxide radicals followed by distillation from sodium in the presence of benzophenone ketal. Dimethyl sulphoxide (DMSO), toluene, dioxane and triethylamine were purchased in anhydrous form.

7.2 Synthesis of adenophostin

7.2.1 2'-*O*-*p*-Methoxybenzyl adenosine (**30**)

To a suspension of dry adenosine (10.02g, 0.038mol) in dry DMF (300cm^3) was added sodium hydride (1.95g of a 60%$^{w}/_{w}$ dispersion in mineral oil, 0.049mol). This suspension was stirred at room temperature for 45 min, after which time *p*-methoxybenzyl chloride (6.23cm^3, 0.045mol) was added over 60 min. Stirring continued for 48 h, when TLC (DCM/methanol 9:1) indicated no further conversion of starting material (R_f 0.11) to product (R_f 0.26). Water (40cm^3) was added, and stirring was continued for 10 min. The resulting yellow solution was concentrated to an oil and subjected to flash chromatography, (being preadsorbed to silica, for application to the column; eluent ethyl acetate then ethyl acetate/ethanol 4:1). To give crude product. Crystallisation from ethanol/water gave pure title compound (4.67g, 32%).
mp 153°C (water/ethanol) (lit.[123]155–156°C); ^{1}H NMR (400 MHz; d_6 DMSO) δ_H 3.55 (1H, m, H-5'$_A$), 3.65 (1H, m, H-5'$_B$), 3.67 (3H, s, OCH_3), 4.02 (1H, m, H-4'), 4.33 (1H, dd, obscured by OCH_2Ar, H-3'), 4.35, 4.56 (2H, AB, J_{AB} 11.7, OCH_2Ar), 4.52 (1H, dd, *J* 5.7, *J* 5.6, H-2'), 5.32 (1H, d, *J* 5.4, D_2O exch., 3'-OH), 5.51 (1H, t, *J* 6.8, D_2O exch., 5'-OH), 6.02 (1H, d, *J* 6.3, H-1'), 6.70 (2H, m, H-3 and H-5 of PMB ring), 7.04 (2H, m, H-2 and H-6 of PMB ring), 7.36 (2H, s, D_2O exch., NH_2), 8.07, 8.30 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 54.95 (q, OCH_3), 61.56 (t, C-5'), 68.98 (d, C-3'), 70.72 (t, OCH_2Ar), 79.73 (d, C-2'), 86.30(d, C-4'), 86.65(d, C-1'), 113.33 (d, C-3 and C-5 of PMB ring), 119.40 (s, C-5), 129.06 (d, C-2 and C-6 of PMB ring), 129.62 (s, C-1 of PMB ring), 139.74 (d, C-8), 148.83 (s, C-4), 152.30 (d, C-2), 156.14 (s, C-6), 158.67 (s, C-4 of PMB ring).

7.2.2 N^6-Benzoyl-2'-*O*-*p*-methoxybenzyl adenosine (**31**)

To a solution of **30** (1.15g, 2.97mmol) in dry pyridine (15cm^3) was added benzoyl chloride (1.85cm^3, 15.76mmol). The resultant rose-coloured solution was

stirred at 0°C for 3 h, when TLC (ethyl acetate) indicated conversion to an intermediate (R_f 0.71). The reaction was quenched with iced water ($20cm^3$) and extracted with DCM ($3 \times 50cm^3$). The combined organic extracts were washed with $50cm^3$ each of water and saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), filtered and concentrated. The resulting yellow solid was redissolved in ethanol ($30cm^3$) and pyridine ($20cm^3$), and this solution was treated with a mixture of 1M aqueous NaOH solution ($40cm^3$) and ethanol ($20cm^3$). The mixture was stirred for 3 min at room temperature and was then concentrated. Methanol ($200cm^3$) was added to the remaining solid, and carbon dioxide was bubbled through this solution, whereupon a white precipitate formed. The solvent was evaporated in *vacuo*, water was added ($100cm^3$) and evaporated *in vacuo*. The resulting solid was extracted with DCM ($3 \times 100cm^3$). The organic extracts were filtered and concentrated. Crystallisation from hot methanol gave the title compound (0.96g, 66%).

mp 156°C (methanol) (lit.[123] 186–187°C); ^{1}H NMR (400 MHz; d_6 DMSO) δ_H 3.56–3.72 (2H, m, H-5'$_A$, H-5'$_B$), 3.68 (3H, s, OCH_3), 4.05 (1H, ddd, *J* 3.7 Hz, 7.1, H-4'), 4.39 (1H, dd, *J* 4.9, 8.3, H-3'), 4.44, 4.63 (2H, AB, J_{AB} 11.7, O*C*H_2Ar), 4.60 (1H, dd, obscured by O*C*H_2Ar, H-2'), 5.20 (1H, t, *J* 5.6, D_2O exch., 5'-OH), 5.39 (1H, d, *J* 5.4, D_2O exch., 3'-OH), 6.19 (1H, d, *J* 6.4, H-1'), 6.75 (2H, m, H-3 and H-5 of PMB ring), 7.10 (2H, m, H-2 and H-6 of PMB ring), 7.56 (2H, m, H-3 and H-5 of Bz ring), 7.65 (1 H, m, H-4 of Bz ring), 8.06 (2H, m, H-2 and H-6 of Bz ring), 8.67, 8.71 (2H, 2s, H-2 and H-8), 11.23 (1H, br. s, D_2O exch., *H*NC=O); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 54.97 (q, OCH_3), 61.21 (t, C-5'), 68.83 (d, C-3'), 70.85 (t, O*C*H_2Ar), 79.92 (d, C-2'), 86.08 (d, C-4'), 86.46 (d, C-1'), 113.44 (d, C-3 and C-5 of PMB ring), 125 84 (s, C-5), 128.51 (d, C-2 and C-6 of PMB ring), 129.13 (d, C-3, C-4 and C-5 of Bz ring), 129.62 (s, C-1 of PMB ring), 132.49 (d, C-2 and C-6 of Bz ring), 133.37 (s, C-1 of Bz ring), 143.01 (d, C-8), 150.44 (s, C-4), 151.51 (d, C-2), 151.90 (s, C-6), 158.70 (s, C-4 of PMB ring), 165.67 (s, *C*OPh).

7.2.3 Attempted preparation of N^6-benzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine (**32**)

31 (0.78g, 1.60mmol) In DMF (15cm^3) was cooled to -5°C and sodium hydride (0.08g of a 60%$^{w}/_{w}$ dispersion in mineral oil, 1.91mmol) was added. After stirring for 50 min at -5°C, benzyl bromide was added dropwise. The reaction mixture was left stirring at -5°C for a further 6.5 h before being quenched with water (10cm^3). Concentration of the reaction mixture gave a yellow oil which was preadsorbed to silica and then subjected to flash chromatography (eluent ethyl acetate/hexane 1:1, then 4:1, then ethyl acetate/ethanol 9:1) to yield a minor product **33** (0.21g, 30%), and a major product **34** (0.61g, 66%).

Data for **33** which was tentatively identified as N^6-benzoyl-N^1-benzyl-2'-*O*-*p*-methoxybenzyl adenosine : R_f 0.34 (ethyl acetate); (Found: $(M+H)^+$, 582.236. Calc for $C_{32}H_{31}N_5O_6$ $[M+H]^+$: 582.235); ^{1}H NMR (400 MHz; d_6 DMSO) δ_H 3.50, 3.58 (2H, m, simplifies to ABX on D_2O exch., $^2J_{AB}$ 12.2, $^3J_{AX} = {}^3J_{BX}$ 3.7, H-5'$_A$, H-5'$_B$), 3.64 (3H, s, OCH_3), 3.98 (1H, m, H-4'), 4.24–4.26 (2H, m, H-3', OC*H*HAr), 4.40–4.47 (2H, m, H-2', OCH*H*Ar), 5.07 (1H, dd, *J* 5.4, D_2O exch., 5'-OH), 5.33 (1H, d, *J* 5.4, D_2O exch., 3'-OH), 5.50 (2H, s, NCH_2Ar), 6.05 (1H, d, *J* 5.9, H-1'), 6.67 (2H, m, H-3 and H-5 of PMB ring), 6.98 (2H, m, H-2 and H-6 of PMB ring), 7.16–7.43 (10H, m, ArCH), 8.54 (2H, s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 51.29 (t, N*C*H$_2$Ar), 55.05 (q, OCH_3), 62.92 (t, C-5'), 70.45 (d, C-3'), 70.45 (d, C-3'), 72.52 (t, O*C*H$_2$Ar), 78.90 (d, C-2'), 87.72 (d, C-4'), 89.03 (d, C-1'), 113.61 (d, C-3 and C-5 of PMB ring), 127.18, 127.36, 127.80, 128.01, 128.28, 128.65, 129.45, 130.89 (8d and 2s, ArCH, C-1 of PMB ring, C-5), 135.83 (s, C-1 of Bz ring), 137.35 (s, C-1 of Bn ring), 143.14 (d, C-8), 150.90 (d and s, C-2 and C-4), 154.69 (s, C-6), 159.42 (s, C-4 of PMB ring), 172.13 (s, *C*OPh); UV ($CHCl_3$) λ_{max} 254 nm; m/z (FAB$^+$) 582 [$(M+1)^+$, 100%].

Data for **34** which was tentatively identified as N^6-benzoyl-N^6-benzyl-2'-*O*-*p*-methoxybenzyl adenosine: R_f 0.15 (ethyl acetate); (Found: $(M+H)^+$, 582.236. Calc for $C_{32}H_{31}N_5O_6$ $[M+H]^+$: 582.235); ^{1}H NMR (400 MHz; d_6 DMSO) δ_H 3.51, 3.58 (2H, m, simplifies to ABX on D_2O exch., $^2J_{AB}$ 12.2, $^3J_{AX}$ 3.9, $^3J_{BX}$ 3.7, H-5'$_A$, H-5'$_B$), 3.64 (3H, s, OCH_3), 3.97–3.99 (1H, m, H-4'), 4.25–4.27 (1H, m, H-3'), 4.32–4.37 (2H, m, H-2', OC*H*HAr), 4.55 (1H, AB, J_{AB} 11.9, OCH*H*Ar), 5.04 (1H, dd, *J* 5.9, D_2O exch., 5'-OH), 5.32 (1H, d, *J* 5.4 D_2O exch., 3'-OH), 5.31, 5.35 (2H, AB J_{AB} 15.0, NCH_2Ar), 5.90 (1H, d, *J* 6.1, H-1'), 6.69–6.71 (2H, m, H-3 and H-5 of PMB ring), 7.01–7.04 (2H, H-2 and H-6 of PMB ring), 7.23–7.46 (8H, m, ArCH), 7.58–7.60 (2H, m, ArCH), 8.04, 8.56 (2H,

2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 52.05 (t, NCH_2Ar), 55.41 (q, OCH_3), 62.89 (t, C-5'), 70.12 (d, C-3'), 72.53 (t, OCH_2Ar), 79.52 (d, C-2'), 87.47 (d, C-4'), 88.72 (d, C-1'), 113.92 (d, C-3 and C-5 of PMB ring), 123.65 (s, C-5), 128.02, 128.33, 128.47, 129.02, 129.63, 129.79, 132.00 (7d and s, ArCH and C-1 of PMB ring), 134.97, 135.41 (2s, C-1 of Bz ring, C-1 of Bn ring), 139.92 (d, C-8), 143.45, 145.79 (2s, C-4, C-6), 146.58 (d, C-2), 159.62 (s, C-4 of PMB ring), 176.99 (s, *C*OPh); UV ($CHCl_3$) λ_{max} 284 nm, 257 nm; m/z (FAB^+) 582 [$(M+1)^+$, 100%].

7.2.4 N^6-Benzoyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-monomethoxytrityl adenosine (**35**)

31 (1.56g, 3.18mmol) Was concentrated repeatedly (four times) from dry pyridine ($10cm^3$) and redissolved in dry pyridine ($25cm^3$). Monomethoxytrityl chloride (1.47g, 4.77mmol), and diisopropylamine ($2.22cm^3$, 12.71mmol) were added to this solution, and stirring was continued overnight at 50°C, after which time TLC (ethyl acetate/ethanol 20:1) indicated no further conversion of starting material to product (R_f 0.65). $20cm^3$ Each of 5% aqueous $NaHCO_3$ solution and methanol were added to quench the reaction. The aqueous layer was extracted with DCM ($3 \times 75cm^3$) and the organic layers were combined and concentrated to a dark yellow oil. This oil was subjected to flash chromatography (eluent ethyl acetate/hexane 4:1) to give the title compound as a clear yellow oil (2.29g, 94%).

$[\alpha]_D$ +1.57 (*c* 5.1, $CHCl_3$); (Found: $(M+H)^+$, 764.310. Calc for $C_{45}H_{42}N_5O_7$ $(M+H)^+$: 764.308); ^1H NMR (400 MHz; d_6 DMSO) δ_H 3.26 (2H, d, *J* 4.4, H-5'$_A$, H-5'$_B$), 3.69, 3.73 (6H, 2s, 2 × OCH_3), 4.17 (1 H, ddd, *J* 4.6, 9.0, H-4'), 4.47–4.52 (2H, m, H-3', OC*H*HAr), 4.66 (1H, AB, *J* 11.7, OCH*H*Ar), 4.73 (1H, dd, *J* 5.1, 5.1, H-2'), 5.40 (1H, d, *J* 5.9, D_2O exch., 3'-OH), 6.21 (1H, d, *J* 4.9, H-1'), 6.76–6.78 (2H, m, H-3 and H-5 of *p*-OCH_3 phenyl ring), 6.84–6.87 (2H, m, H-3 and H-5 of *p*-OCH_3 phenyl ring), 7.13–7.37 (14H, m, ArCH), 7.53–7.57 (2H, m, H-3 and H-5 of Bz ring), 7.63–7.67 (1H, m, H-1 of Bz ring), 8.06 (2H, m, H-2 and H-6 of Bz ring), 8.53, 8.60 (2H, 2s, H-2 and H-8), 11.22 (1H, br. s, D_2O exch., *H*NC=O); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 54.95, 55.01 (2q, OCH_3), 63.55 (t, C-5'), 69.09 (d, C-3'), 71.01 (t, OCH_2Ar), 79.08 (d, C-2'), 83.87 (d, C-

4'), 85.77 (s, MMTr Cq), 86.44 (d, C-1'), 113.16, 113.46 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 125.87 (s, C-5), 126.84, 127.81, 127.92, 128.45, 128.49, 129.18 (6d, ArCH), 129.62 (s, C-1 of PMB ring), 130.01, 132.44 (2d, ArCH), 133.34 (s, C-1 of Bz ring), 134.87 (s, C-1 of MMTr *p*-OCH_3 phenyl ring), 143.25 (d, C-8), 144.09, 144.25 (2s, 2 × C-1 of MMTr phenyl rings), 150.40 (s, C-4), 151.51 (d, C-2), 151.86 (s, C-6), 158.15, 158.74 (2s, C-4 of *p*-OCH_3 phenyl rings), 165.61 (s, *C*OPh); *m/z* (FAB^+) 764 [$(M+H)^+$, 20%], 273 (100).

7.2.5 2',3'-*O*-Benzylidene adenosine (**159**)

Method A

A suspension of zinc chloride (7.00g, 50mmol) in benzaldehyde (35cm^3) was stirred at 5°C for 30 min. Adenosine (2.50g, 9.00mmol) was added and the mixture was stirred in the dark for 7 days at 5°C. The resulting viscous cream-coloured suspension was poured into 500cm^3 of chilled water and this mixture was extracted with ethyl acetate (3 × 200cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. Diisopropyl ether (50cm^3) was added to this mixture of product and benzaldehyde which was then refrigerated overnight. The product was collected by filtration to give the *endo* isomer (3.07g, 92%).

^{1}H NMR (270 MHz; d_6 DMSO) δ_H 3.52–3.63 (2H, m, H-5'$_A$, H-5'$_B$), 4.37–4.41 (1H, m, H-4'), 5.10 (1H, dd, *J* 2.3, 6.5, H-3'), 5.30 (1H, dd, *J* 5.4, D_2O exch., 5'-OH), 5.52 (1H, dd, *J* 2.8, 6.5, H-2'), 6.03 (1H, s, benzylidene C*H*), 6.31 (1H, d, *J* 2.9, H-1'), 7.41–7.60 (5H, m, ArCH), 8.18–8.40 (2H, 2s, H-2, H-8).

Method B

A suspension of zinc chloride (7.00g, 50mmol) in benzaldehyde (35cm^3) was stirred at 5°C for 30 min, before adding adenosine (2.50g, 9.00mmol). This mixture was stirred for 16 h in the dark and then for 1 h at 100°C. An identical work-up to Method A gave 2.10g (63%) of the title compound which was shown by ^{1}H NMR to be a 2:3 ratio of *endo* : *exo* diastereoisomers.

^{1}H NMR (270 MHz; d_6 DMSO) δ_H 3.57–3.63 (2H, m, H-5'$_A$, H-5'$_B$), 4.30–4.32 (0.6H, m, H-4'$_{exo}$), 4.39–4.40 (0.4H, m, H-4'$_{endo}$), 5.09–5.12 (1H, m, H-3'), 5.19 (0.6H, dd, *J* 5.4,

D_2O exch., 5'-OH_{exo}), 5.31 (0.4H, dd, *J* 5.4, D_2O exch., 5'-OH_{endo}), 5.48–5.53 (1H, m, H-2'), 6.03 (0.4H, s, benzylidene CH_{endo}), 6.26 (0.6H, s, benzylidene CH_{exo}), 6.30–6.31 (1H, m, H-1), 7.42–7.56 (5H, m, ArCH), 8.19, 8.40 (2H, 2s, H-2, H-8).

7.2.6 2',3'-*O*-*p*-Methoxybenzylidene adenosine (**36**)

Method A

A suspension of zinc chloride (70g, 0.50mol) in *p*-methoxybenzaldehyde (350cm^3) was stirred for 30 min. Adenosine (25.0g, 0.09mol) was added and the mixture was stirred for 18 h. The resulting viscous cream-coloured suspension was divided into two. Each half was poured into 400cm^3 of chilled water and this mixture was extracted with chloroform (400cm^3, 300cm^3, 200cm^3). The combined organic extracts were washed with water (300cm^3), dried ($MgSO_4$), filtered and concentrated. The suspensions from each half, containing product and *p*-methoxybenzaldehyde, were recombined and left at 4°C overnight. Precipitated product was recovered by filtration (13.8g, endo isomer by ^1H NMR), and remaining product left in the *p*-methoxybenzaldehyde filtrate was precipitated by addition of diisopropyl ether (300cm^3), and the refrigeration (10.0g mixture of *endo* and *exo* isomers, 2:1 *endo* : *exo*, as indicated by the integrals of the *p*-methoxybenzylidene C*H*'s in the ^1H NMR). Combined yield (23.80g, 66%).

R_f 0.26 (ethyl acetate/ethanol, 9:1)

Data for *endo* diastereoisomer: mp 215–217°C (ethanol) (lit.[133] 198–200°C); (Found: C, 55.90; H, 4.87; N 18.10. Calc for $C_{18}H_{19}N_5O_5$: C, 56.08; H, 4.97; N, 18.18%); ^1H NMR (400 MHz; d_6 DMSO) δ_H 3.65–3.63 (2H, m which simplifies on D_2O exch., H-5'$_A$, H-5'$_B$), 3.77 (3H, s, OCH_3), 4.35–4.39 (1H, m, H-4'), 5.06 (1H, dd, *J* 2.2, 6.6, H-3'), 5.28 (1H, t, *J* 5.4, D_2O exch., 5'-OH), 5.47 (1H, dd, *J* 2.9, 6.3, H-2'), 5.96 (1H, s, *p*-methoxybenzylidene C*H*), 6.30 (1H, d, *J* 2.9, H-1'), 6.99 (2H, m, H-3 and H-5 of *p*-methoxybenzylidene phenyl ring), 7.38 (2H, s, D_2O exch., NH_2), 7.50 (2H, m, H-2 and H-6 of *p*-methoxybenzylidene phenyl ring), 8.17, 8.38 (2H, 2s, H-2 and H-8); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 55.83 (q, OCH_3), 62.05 (t, C-5'), 83.12 (d, C-3'), 84.32 (d, C-2'), 86.79 (d, C-4'), 90.30 (d, C-1'), 107.29 (d, *p*-methoxybenzylidene *C*H), 114.44 (d, C-3 and C-5 of *p*-methoxybenzylidene phenyl ring), 119.38 (s, C-5), 128.44 (s, C-1 of *p*-methoxybenzylidene phenyl ring), 129.24 (d, C-2 and C-6 of *p*-methoxybenzylidene

phenyl ring), 140.67 (d, C-8), 149.34 (s, C-4), 153.36 (d, C-2), 156.36 (s, C-6), 161.02 (s, C-4 of *p*-methoxybenzylidene phenyl ring); *m/z* (FAB^+) 386 [$(M+1)^+$, 100%].

Method B

A mixture of *p*-methoxybenzaldehyde ($400cm^3$), adenosine (20.0g, 0.07mol), triethyl orthoformate ($100cm^3$) and dry *p*TSA (40.3g, 0.26mol) was stirred at 35–40°C for 4 h, after which time the resulting purple solution was poured into 0.3M aqueous $NaHCO_3$ solution ($1000cm^3$) and stirred vigorously for 15 min. Ethyl acetate ($300cm^3$) was added and the resulting organic layer was set aside. The aqueous layer was extracted with more ethyl acetate ($300cm^3$) and the combined organic layers were washed with brine ($200cm^3$), dried ($MgSO_4$), filtered and concentrated. Diisopropyl ether ($400cm^3$) was added and the suspension was kept at 4°C for 48 h. The product was isolated as a white powder (26.01g as a mixture of *endo* and *exo* isomers, 2:3 *endo* : *exo*, by ^1H NMR, 90%).

Data for mixture of diastereoisomers: ^1H NMR (400 MHz; d_6 DMSO) δ_H 3.57–3.62 (2H, m, H-5'$_A$, H-5'$_B$), 3.77 (1.8H, s, OCH_{3exo}), 3.79 (1.2H, s, OCH_{3endo}), 4.26–4.31 (0.6H, m, H-4'$_{exo}$), 4.37–4.40 (0.4 H, m, H-4'$_{endo}$), 5.05–5.10 (1H, m, H-3'), 5.16 (0.6H, t, *J* 5.6, D_2O exch., 5'-OH_{exo}), 5.29 (0.4H, t, *J* 5.6, D_2O exch., 5'-OH_{endo}), 5.45–5.49 (1H, m, H-2'), 5.97 (0.4H, s, *p*-methoxybenzylidene CH_{endo}), 6.19 (0.6H, s, *p*-methoxybenzylidene CH_{exo}), 6.28 (0.6H, d, *J* 2.9, H-1'$_{exo}$), 6.30 (0.4H, d, *J* 2.9, H-1'$_{endo}$), 6.95–7.02 (2H, m, H-3 and H-5 of *p*-methoxybenzylidene phenyl ring), 7.39–7.52 (4H, m, H-2 and H-6 of *p*-methoxybenzylidene ring, D_2O exch., NH_2), 8.18, 8.39 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 55.21 (q, OCH_3), 61.57 (t, C-5'), 80.47, 82.81, 84.45 (3d, C-4', C-3', C-2' all *exo*), 82.59, 83.67, 86.30 (3d, C-4', C-3', C-2' all *endo*), 87.98 (d, C-1'$_{exo}$), 89.55 (d, C-1'$_{endo}$), 102.87 (d, *p*-methoxybenzylidene CH_{exo}), 106.56 (d, *p*-methoxybenzylidene CH_{endo}), 113.66 (d, C-3 and C-5 of *p*-methoxybenzylidene phenyl ring$_{exo}$), 113.79 (d, C-3 and C-5 of *p*-methoxybenzylidene phenyl ring$_{endo}$), 119.14 (s, C-5), 128.07 (s, C-1 of *p*-methoxybenzylidene phenyl ring), 128.51, (d, C-2 and C-6 of *p*-methoxybenzylidene phenyl ring), 139.72 (d, C-8$_{exo}$), 139.88 (d, C-8$_{endo}$), 148.81 (s, C-4), 152.68 (d, C-2), 156.18 (s, C-6), 160.26 (s, C-4 of *p*-methoxybenzylidene phenyl ring$_{exo}$), 160.38 (s, C-4 of *p*-methoxybenzylidene phenyl ring$_{endo}$).

7.2.7 N^6-Dimethoxytrityl-2',3'-*O*-*p*-methoxybenzylidene adenosine (**37**)

36 (3.83g, 8.78mmol) Was repeatedly concentrated from dry pyridine (3 × 20cm^3), and resuspended in dry pyridine (50cm^3). After addition of chlorotrimethylsilane (2.79cm^3, 21.95mmol) the starting material dissolved. Stirring was continued for 2 h before dimethoxytrityl chloride (7.83g, 21.95mmol) was added. The resultant orange mixture was stirred overnight. The mixture was cooled to 0°C and the reaction was quenched by addition of water (5cm^3). The cooling bath was removed and after 10 min concentrated aqueous ammonia (20cm^3) was added. Stirring was continued for a further 30 min, then the solution was partitioned between 5% aqueous $NaHCO_3$ solution (150cm^3) and DCM (150cm^3). The aqueous layer was extracted with DCM (2 × 50cm^3) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated to leave a dark orange oil that was subjected to flash chromatography (eluent ethyl acetate/hexane 3:2) to give the title compound as a pale cream foam (6.60g, 97%).

R_f 0.29 (ethyl acetate/hexane 3:2)

Data for *endo* diastereoisomer: (Found: M^+, 687.267. Calc for $C_{39}H_{37}N_5O_7$ M^+: 687.269); ^{1}H NMR (400 MHz; d_6 DMSO) δ_H 3.49–3.61 (2H, m, H-5'$_A$, H-5'$_B$), 3.71 (6H, s, 2 × OCH_3 of DMTr), 3.78 (3H, s, OCH_3 of *p*-methoxybenzylidene), 4.35–4.39 (1H, m, H-4'), 5.03 (1H, dd, *J* 2.3, 6.5, H-3'), 5.22 (1H, t, *J* 6.6, D_2O exch., 5'-OH), 5.48 (1H, dd, *J* 2.8, 6.5, H-2'), 5.95 (1H, s, *p*-methoxybenzylidene C*H*), 6.30 (1H, d, *J* 2.8, H-1'), 6.82–6.86 (4H, m, ArCH), 6.98–7.01 (2H, m, ArCH), 7.19–7.29 (9H, m, ArCH), 7.48 (1H, br. s, D_2O exch., NH), 7.51–7.96 (2H, m, ArCH), 8.32, 8.48 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 55.36 (q, 2 × OCH_3), 55.58 (q, OCH_3), 61.76 (t, C-5'), 69.93 (s, DMTr Cq), 82.84, 84.01, 86.70 (3d, C-4', C-3', C-2'), 90.01 (d, C-1'), 106.95 (d, *p*-methoxybenzylidene *C*H), 113.35, 114.15 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 120.97 (s, C-5), 126.86, 128.07, 128.34, 128.71, 128.91, 130.12 (5d, ArCH, and s, C-1 of *p*-methoxybenzylidene phenyl ring), 137.52 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 140.85 (d, C-8), 145.57 (s, C-1 of DMTr phenyl ring), 148.31 (s, C-4), 151.79 (d, C-2), 153.97 (s, C-6), 158.04 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 160.75 (s, C-4 of *p*-methoxybenzylidene phenyl ring); *m/z* (FAB$^+$) 688 [(M+H)$^+$, 26%], 303 (100).

Data for mixture of diastereoisomers, *endo* : *exo* 2:3: ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.77–3.84 (1H, m, H-5'$_{Aexo}$, H-5'$_{Aendo}$), 3.77 (6H, s, 2 × OCH_3 of DMTr), 3.80 (3H, s, OCH_3 of *p*-methoxybenzylidene), 3.94–4.00 (1H, m, H-5'$_B$), 4.53 (0.4H, m, H-4'$_{exo}$), 4.68 (0.6H, m, H-4'$_{endo}$), 5.16–5.18 (1H, m, H-3'), 5.26–5.35 (1H, m, H-2'), 5.98 (0.4H, d, *J* 4.95, H-1'$_{endo}$), 6.00–6.02 (1H, m, *p*-methoxybenzylidene C*H*$_{endo}$, H-1'$_{exo}$), 6.25 (0.6H, s, *p*-methoxybenzylidene C*H*$_{exo}$), 6.77–7.02 (7H, m, ArCH), 7.21–7.50 (10H, m, ArCH), 7.76, 7.83 (1H, 2s, H-2 or H-8), 8.01, 8.02 (1H, 2s, H-2 or H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.59 (q, OCH_3), 63.41 (t, C-5'$_{exo}$), 63.72 (t, C-5'$_{endo}$), 71.12 (s, DMTr Cq), 80.52, 84.21, 86.43 (3d, C-2', C-3', C-4' all *endo*), 83.07, 84.01, 85.91 (3d, C-2', C-3', C-4' all *exo*), 92.26 (d, C-1'$_{exo}$), 94.47 (d, C-1'$_{endo}$), 104.93 (d, *p*-methoxybenzylidene C*H*$_{exo}$), 107.85 (d, *p*-methoxybenzylidene C*H*$_{endo}$), 113.41, 114.15 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 122.43 (s, C-5$_{exo}$), 122.56 (s, C-5$_{endo}$), 127.12, 127.97, 128.14, 128.28, 128.83, 128.93, 130.27 (6d, ArCH, and s, C-1 of *p*-methoxybenzylidene phenyl ring), 137.30 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 139.49, (d, C-8$_{exo}$)139.81 (d, C-8$_{endo}$), 145.28 (s, C-1 of DMTr phenyl ring), 147.31 (s, C-4$_{endo}$), 147.45 (s, C-4$_{exo}$), 152.02 (d, C-2$_{endo}$), 152.20 (d, C-2$_{exo}$), 154.73 (s, C-6$_{exo}$), 154.76 (s, C-6$_{endo}$), 158.46 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 160.79 (s, C-4$_{endo}$ of *p*-methoxybenzylidene phenyl ring), 161.00 (s, C-4$_{exo}$ of *p*-methoxybenzylidene phenyl ring).

7.2.8 5'-*O*-Benzyl-*N*^{6}-dimethoxytrityl-2',3'-*O*-*p*-methoxybenzylidene adenosine (**38**)

Potassium Hydroxide 85% (14.57g, 220.69mmol) and benzyl chloride (3.05cm^3, 26.48mmol) were consecutively added to **37** (6,06g, 8.83mmol) in benzene (85cm^3) and dioxane (41cm^3). The reaction mixture was refluxed for 20 min, cooled and partitioned between ether (200cm^3) and iced water (150cm^3). The ethereal layer was washed with water (2 × 75cm^3), and the combined aqueous layers were extracted with ether (100cm^3). The combined ethereal layers were dried ($MgSO_4$), filtered and concentrated and the remaining yellow oil subjected to flash chromatography (eluent ethyl acetate/hexane 1:1) to yield the title compound as a pale cream foam (6.53g, 95%).

Data for *endo* diastereoisomer: (Found: C, 70.80; H, 5.53; N, 8.85. Calc for $C_{46}H_{43}N_5O_7$: C, 71.03; H, 5.57; N, 9.00%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.68–3.72 (2H, m, H-5'$_A$, H-5'$_B$), 3.77 (6H, s, 2 × OCH_3 of DMTr), 3.82 (3H, s, OCH_3 of *p*-methoxybenzylidene), 4.46, 4.49 (2H, AB, J_{AB} 12.2, O*CH*$_2$Ar), 4.65 (1H, ddd, *J* 2.4, H-4'), 5.04 (1H, dd, *J* 2.4, 6.4, H-3'), 5.51 (1H, dd, *J* 2.2, 6.6, H-2'), 5.95 (1H, s, *p*-methoxybenzylidene C*H*), 6.27 (1H, d, *J* 2.5, H-1'), 6.78–6.94 (7H, m, ArCH), 7.20–7.48 (15H, m, ArCH), 7.96, 8.05 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.21 (q, 2 × OCH_3), 55.34 (q, OCH_3), 70.10 (t, C-5'), 70.64 (s, DMTr Cq), 73.45 (t, O*CH*$_2$Ar), 82.79, 84.86, 85.57 (3d, C-2', C-3', C-4'), 90.89 (C-1'), 107.66, (d, *p*-methoxybenzylidene *C*H), 113.13, 113.89 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 121.32 (s, C-5), 126.81, 127.72, 127.78, 127.89, 128.14, 128.31, 128.47, 128.78, 130.10 (8d, ArCH, and s, C-1 of *p*-methoxybenzylidene phenyl ring), 137.31 (s, C-1 of Bn ring), 137.45 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 138.81 (d, C-8), 145.43 (s, C-1 of DMTr phenyl ring), 148.39 (s, C-4), 152.47 (d, C-2), 154.15 (s, C-6), 158.26 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 160.88 (s, C-4 of *p*-methoxybenzylidene phenyl ring); *m/z* (FAB$^+$) 688 [(M+H)$^+$, 26%], 303 (100).

Data for mixture of diastereoisomers, *endo* : *exo* 2:3: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.67 (0.4H, 0.5 ABX, $^2J_{AB}$ 13.9, $^3J_{AX}$ 4.2, H-5'$_{Aendo}$), 3.68–3.72 (1.6H, m, H-5'$_{Aexo}$, H-5'$_B$), 3.77, 3.81, 3.82 (6H, s, 2 × OCH_3 of DMTr), 3.81, 3.82 (3H, 2s, OCH_3 of *p*-methoxybenzylidene), 4.46 (0.4H, AB, J_{AB} 12.2, OC*H*HAr$_{endo}$), 4.48–4.54 (2.2H, m, OCH$_2$Ar$_{exo}$, OCH*H*Ar$_{endo}$, H-4'$_{exo}$), 4.65 (0.4H, ddd, *J* 2.4, H-4'$_{endo}$), 5.04 (0.4H, dd, *J* 2.4, 6.4, H-3'$_{endo}$), 5.13 (0.6H, dd, *J* 3.7, 6.1, H-3'$_{exo}$), 5.45 (0.6H, dd, *J* 2.9, 6.4, H-2'$_{exo}$), 5.51 (0.4H, dd, *J* 2.2, 6.6, H-2'$_{endo}$), 5.95 (0.4H, s, *p*-methoxybenzylidene C*H*$_{endo}$), 6.13 (0.6H, s, *p*-methoxybenzylidene C*H*$_{exo}$), 6.21 (0.6H, d, *J* 2.4, H-1'$_{exo}$), 6.27 (0.4H, d, *J*, 2.5, H-1'$_{endo}$), 6.78–6.94 (7H, m, ArCH), 7.20–7.48 (15H, m, ArCH), 7.03, 7.95 (1H, 2s, H-2 or H-8), 7.96, 8.05 (1H, 2s, H-2 or H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.21 (q, 2 × OCH_3), 55.34 (q, OCH_3), 70.10 (t, C-5'), 70.64 (s, DMTr Cq), 73.45 (t, O*CH*$_2$Ar), 81.33, 84.00, 84.27 (3d, C-2', C-3', C-4' all *exo*), 82.79, 84.86, 85.57 (3d, C-2', C-3', C-4' all *endo*), 89.98 (d, C-1'$_{exo}$), 90.89 (d, C-1'$_{endo}$), 104.26 (d, *p*-methoxybenzylidene *C*H$_{exo}$), 107.66, (d, *p*-methoxybenzylidene *C*H$_{endo}$),113.13, 113.89 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 121.32 (s, C-5), 126.81, 127.72, 127.78, 127.89, 128.14, 128.31, 128.47, 128.78, 130.10 (8d, ArCH, and s, C-1 of *p*-methoxybenzylidene phenyl ring), 137.31 (s,

C-1 of Bn ring), 137.45 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 138.81 (d, C-8), 145.43 (s, C-1 of DMTr phenyl ring), 148.39 (s, C-4), 152.47 (d, C-2), 154.15 (s, C-6), 158.26 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 160.88 (s, C-4 of *p*-methoxybenzylidene phenyl ring).

7.2.9 3'-*O*-Acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine (**42**) and 2'-*O*-acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-3'-*O*-*p*-methoxybenzyl adenosine (**41**)

38 (1.00g, 1.29mmol) In DCM (6cm^3) was cooled to -78°C and 1M DIBAL-H in DCM (6.5cm^3, 6.50mmol) was added dropwise. The reaction mixture was allowed to warm slowly to -25°C over 2 h, after which time TLC (ethyl acetate/pentane 1:1) indicated conversion to two close-running products R_f 0.51 (minor product) and 0.39 (major product). The reaction mixture was again cooled to -78°C and quenched with ethyl acetate (5cm^3) at -78°C. Ether (100cm^3) was added and the cooling bath was removed. Ice-cold 1M NaOH solution was added until there were two clear layers, the resulting aqueous layer was discarded. The organic layer was washed with water (75cm^3), dried ($MgSO_4$), filtered and concentrated to a yellow oil that was directly acetylated by dissolving in pyridine (10cm^3) and acetic anhydride (5cm^3) and leaving to stir overnight. The reaction mixture was concentrated, and then concentrated repeatedly from toluene. The residue was subjected to flash chromatography (eluent ethyl acetate /hexane 2:3, then 1:1, then 3:2). Initial fractions contained pure 3-*O*-acetyl-5-*O*-benzyl-N^6-dimethoxytrityl-2',*O*-*p*-methoxybenzyl adenosine, while further fractions contained a mixture of the two regioisomers. The material in these fractions was repeatedly recolumned in order to completely separate the two regioisomers. Yield of 3'-*O*-acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine (0.58g, 55% over two steps). Yield of 2'-*O*-acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-3'-*O*-*p*-methoxybenzyl adenosine (0.39g, 38% over 2 steps).

Data for 3'-*O*-acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine (**42**): R_f 0.35 (chloroform/acetone 19:1); (Found: C, 70.00; H, 5.80; N, 8.49. Calc for $C_{48}H_{47}N_5O_8$: C, 70.14; H, 5.76; N, 8.52%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 2.14 (3H, s,

CH_3CO), 3.67 (1H, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.1, H-5'$_A$), 3.74–3.79 (1H, m, H-5'$_B$, obscured by OCH_3), 3.75 (3H, s, OCH_3), 3.76 (6H, s, 2 × OCH_3), 4.34–4.39 (2H, AB, J_{AB} 12.0, OC*H*HAr, overlapping with H-4'), 4.50 (1H, AB, J_{AB} 12.0, OCH*H*Ar), 4.54, 4.58 (2H, AB, J_{AB} 11.7, OCH_2Ar), 4.68 (1H, dd, *J* 5.3, 6.4, H-2'), 5.43 (1H, dd, *J* 2.8, 5.1, H-3'), 6.12 (1H, d, *J* 6.7, H-1'), 6.66–6.69 (2H, m, ArCH), 6.79–6.83 (4H, m, ArCH), 6.88 (1H, br.s, D_2O exch., NH), 6.95–6.99 (2H, m, ArCH), 7.22–7.38 (14H, m, ArCH), 7.77, 8.06 (2H, s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 21.36 (q, *C*H_3CO), 55.59 (q, 3 × OCH_3), 69.96 (t, C-5'), 70.96 (s, DMTr Cq), 72.14, 79.01, 82.44 (3d, C-2', C-3', C-4'), 72.86, 74.06 (2t, 2 × O*C*H_2Ar), 86.38 (d, C-1'), 113.36, 113.95 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings),121.20 (s, C-5), 127.00, 128.01, 128.08, 128.23, 128.82, 129.02, 129.73, 130.32 (7d, ArCH, and s, C-1 of PMB ring), 137.49 (s, C-1 of Bn ring), 137.68 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 138.23 (d, C-8), 145.65 (s, C-1 of DMTr phenyl ring), 149.05 (s, C-4), 152.60 (d, C-2), 154.19 (s, C-6), 158.38 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 159.60 (s, C-4 of PMB ring), 170.39 (s, CH_3*C*O); *m/z* (FAB^+) 822 [$(M+H)^+$, 30%], 303 (100), 91 (17).

Data for 2'-*O*-acetyl-5'-*O*-benzyl-N^6-dimethoxytrityl-3'-*O*-*p*-methoxybenzyl adenosine (**41**): R_f 0.30 (chloroform/acetone 19:1); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.54 (1H, 0.5 ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.9, H-5'$_A$), 3.77–3.79 (1H, m, H-5'$_B$, obscured by OCH_3), 3.77 (3H, s, OCH_3), 3.78 (6H, s, 2 × OCH_3), 4.22–4.24 (1H, m, H-4'), 4.34 (1H, AB, J_{AB} 10.7, OC*H*HAr), 4.46–4.58 (4H, m, 3 × OCH*H*Ar, H-3'), 5.72 (1H, dd, *J* 2.9, 4.9, H-2'), 6.17 (1H, dd, *J* 2.9, H-1'), 6.78–6.87 (9H, m, ArCH, D_2O exch., NH), 7.17–7.35 (14H, m, ArCH), 8.05, 8.07 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.81 (q, CH_3CO), 55.23 (q, 3 × OCH_3), 68.40 (t, C-5'), 70.61 (s, DMTr Cq), 72.89, 73.39 (2t, 2 × OCH_2Ar), 74.41, 75.33, 81.55 (3d, C-2', C-3', C-4'), 87.09 (d, C-1'), 113.15, 113.84 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 126.81 (s, C-5), 127.72, 127.87, 128.51, 128.78, 129.30, 129.86, 130.10 (6d, ArCH, and s, C-1 of PMB ring), 137.43 (s, C-1 of Bn ring), 137.53 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 138.57 (d, C-8), 145.11 (s, C-1 of DMTr phenyl ring), 148.00 (s, C-4), 152.49 (d, C-2), 153.66 (s, C-6), 158.26 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 159.66 (s, C-4 of PMB ring), 169.91 (s, CH_3*C*O).

7.2.10 5'-*O*-Benzyl-N^6-dimethoxytrityl-2'-*O*-*p*-methoxybenzyl adenosine (**40**)

A solution of **42** (3.85g, 4.69mmol), in methanol ($60cm^3$) chloroform ($30cm^3$) and concentrated ammonia ($15cm^3$) was stirred in a sealed flask for 48 h. The solvents were removed under reduced pressure and the residue repeatedly concentrated from chloroform and then subjected to flash chromatography (eluent ethyl acetate /hexane 3:2) to yield the title compound as a white foam in quantitative yield (3.65g).

R_f 0.39 (ethyl acetate/hexane 1:1); Found: C, 70.60; H, 5.86; N, 8.91. Calc for $C_{46}H_{45}N_5O_7$: C, 70.84; H, 5.82; N, 8.98%; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.66 (1H, 0.5 ABX, $^2J_{AB}$ 10.8, $^3J_{Ax}$ 3.5, H-5'$_A$), 3.77 (3H, s, OCH_3), 3.78–3.82 (1H, 0.5ABX obscured by OCH_3, $^3J_{BX}$ 2.6, H-5'$_B$), 3.78 (6H, s, 2 × OCH_3), 4.18–4.21 (1H, m, H-4'), 4.34 (1H, dd, *J* 5.0, H-3'), 4.47 (1H, dd, *J* 4.7, H-2'), 4.56, 4.60 (2H, AB, J_{AB} 12.0, OCH_2Ar), 4.57, 4.66 (2H, AB, J_{AB} 11.7, OCH_2Ar), 6.16 (1H, d, *J* 4.1, H-1'), 6.71–6.84 (7H, m, ArCH), 6.93 (1H, br. s, D_2O exch., NH), 7.11–7.38 (15H, m, ArCH), 7.97, 8.07 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.59 (q, 3 × OCH_3), 69.69 (t, C-5'), 70.33, 81.24, 84.20 (3d, C-2', C-3', C-4'), 70.96 (s, DMTr Cq), 72.97, 73.90 (2t, 2 × OCH_2Ar), 87.03 (d, C-1'), 113.35, 114.18 (2d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 121.30 (s, C-5), 127.00, 127.94, 128.06, 128.13, 128.77, 128.82, 129.02, 130.33 (7d, ArCH, and s, C-1 of PMB ring), 137.67 (s, C-1 of DMTr *p*-OCH_3 phenyl rings), 137.72 (s, C-1 of Bn ring), 138.53 (d, C-8), 145.63 (s, C-1 of DMTr phenyl ring), 148.62 (s, C-4), 152.49 (d, C-2), 154.19 (s, C-6), 158.38 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 159.82 (s, C-4 of PMB ring); *m/z* (FAB^+) 780 [$(M+H)^+$, 32%], 303 (100), 91 (21).

7.2.11 5'-*O*-Benzyl adenosine (**43**)

38 (3.46g, 4.45mmol) In 80% aqueous acetic acid ($170cm^3$) was heated at 100°C for 45 min. After cooling the acetic acid was evaporated under reduced pressure and the residue repeatedly concentrated from toluene to a yellow solid which was dissolved in minimum of methanol. Ether was added and the mixture left at -20°C over-night. The title compound was isolated as a white powder by filtration (1.35g, 85%).

(Found: M^+, 358.152. Calc for $C_{17}H_{20}N_5O_4$ $(M+H)^+$: 358.151); ^1H NMR (270 MHz; d_6 DMSO) δ_H 3.64, 3.75 (2H, ABX, $^2J_{AB}$, 10.6, $^3J_{AX}$ 5.0, $^3J_{BX}$ 3.6, H-5'$_A$, H-5'$_B$), 4.08 (1H, ddd, *J* 4.0, 4.2, H-4'), 4.22 (1H, ddd, *J* 4.9, 9.4, H-3'), 4.53 (2H, s, OC*H*$_2$Ar), 4.60 (1H, dd, *J* 5.3, 10.8, H-2'), 5.31 (1H, d, *J* 5.3, -OH), 5.54 (1H, d, *J* 6.1, -OH), 5.92 (1H, d, *J* 5.3, H-1'), 7.26–7.37 (5H, m, ArCH), 8.15, 8.27 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; d_6 DMSO) δ_C 70.10 (t, C-5'), 70.57, 73.52, 83.25 (3d, C-2', C-3', C-4'), 72.42 (t, OCH_2Ar), 87.45 (d, C-1'), 118.95 (s, C-5), 127.52, 128.31 (2d, ArCH), 138.20 (s, C-1 of Bn ring), 139.55 (d, C-8), 149.34 (s, C-4), 151.79 (d, C-2), 155.35 (s, C-6); *m/z* (FAB^+) 358 [$(M+H)^+$, 100%], 91 (37).

7.2.12 *p*-Methoxybenzyl bromide[148] (**160**)

A solution of *p*-methoxybenzyl alcohol (48g, 0.35mol) in ether (75cm^3) was added slowly to a solution of 48%HBr (80g) in ether (75cm^3). After addition the mixture was stirred for a further 30 min and then quenched with an excess of solid $NaHCO_3$ (27g). The resulting two layers were separated and the aqueous layer extracted with ether (2 × 50cm^3). The ethereal extracts were combined with the organic layer, washed with water (100cm^3), dried ($MgSO_4$), filtered and concentrated to a clear liquid. The crude product was distilled [bp 109°C (1mm); lit.[148] 92–95°C (0.02mm)] to give a colourless liquid that was stored over dry K_2CO_3.

7.2.13 5'-*O*-Benzyl-2'-*O*-*p*-methoxybenzyl adenosine (**44**)

43 (258mg, 0.72mmol) In DMF (13cm^3) was cooled to -20°C and sodium hydride (38mg of a 60%^{w}/w dispersion in mineral oil, 0.94mmol) was added. After stirring for one h at -20°C to -12°C *p*-methoxybenzyl bromide in DMF (5cm^3) was added dropwise over 2 h 30 min. The reaction mixture was left stirring for a further 2 h at -20°C before being quenched with water (2cm^3). Concentration of the reaction mixture gave a clear oil that was preadsorbed to silica and then subjected to flash chromatography (eluent ethyl acetate/ethanol 39:1, then 19:1) to yield the title compound (241mg, 70%).

R_f 0.38 (ethyl acetate/ethanol 39:1); (Found: M^+, 478.209. Calc for $C_{25}H_{28}N_5O_5$ $(M+H)^+$: 478.209); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.69, 3.83 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.2, $^3J_{BX}$ 2.8, H-5'$_A$, H-5'$_B$), 3.76 (3H, s, OCH_3), 4.23–4.27 (1H, m, H-4'), 4.39 (1H, dd, *J* 4.8, H-3'), 4.44 (1H, dd, *J* 4.7, H-2'), 4.53–4.65 (4H, m, 2 × O*C*H_2Ar), 6.13 (2H, br. s, D_2O exch., NH_2), 6.20 (1H, d, *J* 4.0, H-1'), 6.70–6.73 (2H, m, H-3 and H-5 of PMB ring), 7.07–7.10 (2H, m, H-2 and H-6 of PMB ring), 7.29–7.36 (5H, m, ArCH), 7.99, 8.24 (2H, 2s, H-2, H-8); ^{13}C NMR (67.9 MHz; $CDCl_3$) δ_C 55.01 (q, OCH_3), 69.17 (t, C-5'), 69.93, 80.91, 83.93 (3d, C-2', C-3', C-4'), 72.31, 73.45 (2t, 2 × O*C*H_2Ar), 86.54 (d, C-1'), 113.62 (d, C-3 and C-5 of PMB ring), 119.43 (s, C-5), 127.55, 127.60, 127.78, 128.33, 128.38, 129.56 (6d, ArCH), 128.44 (s, C-1 of PMB ring), 137.39 (s, C-1 of Bn ring), 138.66 (d, C-8), 149.28 (s, C-4), 152.75 (d, C-2), 155.23 (s, C-6), 159.35 (s, C-4 of PMB ring); *m/z* (FAB^+) 478 $[(M+H)^+$, 100%], 121 (88).

A sample of 5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine was converted to its N^6,3'-*O*-diacetate with acetic anhydride and pyridine in the usual way.
^{1}H NMR (270 MHz; $CDCl_3$) δ_H 2.16, 2.18 (6H, 2s, 2 × CH_3CO), 3.67–3.74 (1H, m, H-5'$_A$, obscured by OCH_3), 3.73 (3H, s, OCH_3), 3.78 (1H, 0.5 ABX, $^2J_{AB}$ 10.4, $^3J_{AX}$ 2.9, H-5'$_B$), 4.31, 4.84 (2H, AB, J_{AB} 11.9, O*C*H_2Ar), 4.36–4.39 (1H, m, H-4'), 4.57 (2H, s, O*C*H_2Ar), 4.64 (1H, dd, *J* 5.1, 6.8, H-2'), 5.46 (1H, dd, *J* 5.1, 2.4, H-3'), 6.13 (1H, d, *J* 6.6, H-1'), 6.19 (1H, br. s, D_2O exch., NH), 6.58–6.61 (2H, m, H-3 and H-5 of PMB ring), 6.88–6.91 (2H, m, H-2 and H-6 of PMB ring), 7.29–7.39 (5H, m, ArCH), 7.78, 8.32 (2H, 2s, H-2, H-8).

7.2.14 N^6-Benzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine (**32**)

After being repeatedly concentrated from dry pyridine **44** (413mg, 0.87mmol) was redissolved in dry pyridine (5cm^3) and chlorotrimethylsilane (0.27cm^3, 2.16mmol) was added. This mixture was stirred for one h before benzoyl chloride (0.50cm^3, 4.33mmol) was added. Stirring was continued for a further 2 h, and then the mixture was cooled to 0°C and the reaction quenched by addition of water (1cm^3). After 5 min concentrated aqueous ammonia (2cm^3) was added and the reaction mixture was allowed

to warm to room temperature over 30 min. Subsequent concentration of the reaction mixture was followed by partitioning of the residue between DCM ($50cm^3$) and saturated aqueous $NaHCO_3$ solution ($30cm^3$). The organic layer was dried ($MgSO_4$), filtered and concentrated and the resulting yellow oil subjected to flash chromatography (eluent ethyl acetate/hexane 9:1) to yield the title compound as a clear oil (323mg, 64%).
R_f 0.60 (ethyl acetate/hexane 9:1); (Found: M^+, 582.236. Calc for $C_{32}H_{32}N_5O_6$ $(M+H)^+$: 582.235); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.12 (1H, br. s, D_2O exch., 3'-OH), 3.66, 3.83 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 2.9, $^3J_{BX}$ 2.4, H-5'$_A$, H-5'$_B$), 3.73 (3H, s, OCH_3), 4.24–4.26 (1H, m, H-4'), 4.37 (1H, dd, *J* 4.4, H-3'), 4.47 (1H, dd, *J* 4.4, H-2'), 4.54–4.63 (4H, m, 2 × OCH_2Ar), 6.24 (1H, d, *J* 4.4, H-1'), 6.67–6.71 (2H, m, H-3 and H-5 of PMB ring), 7.05–7.07 (2H, m, H-2 and H-6 of PMB ring), 7.22–7.39 (5H, m, ArCH), 7.50–7.53 (2H, m, H-3 and H-5 of Bz ring), 7.57–7.61 (1H, m, H-4 of Bz ring), 8.03–8.04 (2H, m, H-2 and H-6 of Bz ring), 8.22, 8.77 (2H, 2s, H-2, H-8), 9.22 (1H, br. s, D_2O exch., NH); ^{13}C NMR (67.9 MHz; $CDCl_3$) δ_C 55.24 (q, OCH_3), 69.33 (t, C-5'), 70.14, 81.14, 84.30 (3d, C-2', C-3', C-4'), 72.81, 73.68 (2t, 2 × OCH_2Ar), 86.91 (d, C-1), 113.86 (d, C-3 and C-5 of PMB ring), 123.14 (s, C-5), 127.80, 127.89, 128.02, 128.61, 128.83, 129.81, 132.74 (7d, ArCH), 128.36 (s, C-1 of PMB ring), 133.78 (s, C-1 of Bz ring), 137.41 (s, C-1 of Bn ring), 141.45 (d, C-8), 149.36 (s, C-4), 151.37 (s, C-6), 152.54 (d, C-2), 159.66 (s, C-4), 164.72 (s, *C*OPh); *m/z* (FAB^+) 582 [$(M+H)^+$, 100%], 240 (40).

7.2.15 N^6,N^6-Dibenzoyl-5'-*O*-benzyl-2'-*O*-*p*-methoxybenzyl adenosine (45)

After being repeatedly concentrated from dry pyridine **44** (428mg, 0.90mmol) was redissolved in pyridine ($7cm^3$) and chlorotrimethylsilane ($0.28cm^3$, 2.24mmol) added. This mixture was stirred for one h then benzoyl chloride ($0.52cm^3$, 4.49mmol) was added and stirring was continued for a further 2 h. The reaction was quenched by cooling the mixture to 0°C and the addition of water ($1cm^3$). The mixture was stirred for a further 30 min at room temperature and then was concentrated and the residue partitioned between DCM ($75cm^3$) and saturated aqueous $NaHCO_3$ solution ($50cm^3$). The organic layer was dried ($MgSO_4$), filtered and concentrated and the resulting yellow

oil was subjected to flash chromatography (eluent ethyl acetate/hexane 3:2) to yield the title compound as a clear oil (539mg, 88%).

R_f 0.40 (ethyl acetate/hexane 3:2); (Found: M^+, 686.261. Calc for $C_{39}H_{36}N_5O_7$ $(M+H)^+$: 686.261); ^1H NMR (270 MHz; $CDCl_3$) δ_H 3.61 (1H, 0.5 ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 3.1, H-5'$_A$), 3.74–3.79 (1H, m, H-5'$_B$, obscured by OCH_3), 3.75 (3H, s, OCH_3), 4.19–4.21 (1H, m, H-4'), 4.32 (1H, dd, *J* 4.9, H-3'), 4.45–4.65 (5H, m, H-2', 2 × OC*H*$_2$Ar), 6.24 (1H, d, *J* 4.2, H-1'), 6.77–6.80 (2H, m, H-3 and H-5 of PMB ring), 7.06–7.10 (2H, m, H-2 and H-6 of ring), 7.23–7.37 (9H, m, ArCH), 7.44–7.50 (2H, m, H-4 of Bz rings), 7.84–7.87 (4H, m, H-2 and H-6 of Bz rings), 8.33, 8.61 (2H, 2s, H-2, H-8); *m/z* (FAB^+) 686 [$(M+H)^+$, 100%], 91 (52).

7.2.16 N^6,N^6-Dibenzoyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-2',3",4"-tri-*O*-*p*-methoxybenzyl adenosine (**48**)

47 (201mg, 0.29mmol) And **45** (100mg, 0.15mmol) in dioxane (2.25cm^3) and toluene (0.75cm^3) were stirred with 4Å molecular sieves (approx. 150mg) for 1.75 h, and then dry zinc chloride (34mg, 0.25mmol) and silver perchlorate (104mg, 0.50mmol) were added. The flask was wrapped in foil and stirring was continued for 44 h. Solid $NaHCO_3$ (200mg) and water (2cm^3) were added and the reaction mixture was diluted with ethyl acetate (5cm^3), after stirring for a further 15 min the mixture was filtered through a celite pad, and the residue was well washed with ethyl acetate. The filtrate was washed with brine (20cm^3) and saturated aqueous $NaHCO_3$ solution (20cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent ethyl acetate/hexane 7:3) to yield the title compound as a clear oil (29mg, 16%).

R_f 0.56 (ethyl acetate/toluene 1:4); (Found: M^+, 1268.520. Calc for $C_{75}H_{73}N_5O_{14}$ $(M+H)^+$: 1268.523); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.51–3.53 (1H, m, H-5'$_A$), 3.57–3.81 (6H, m, H-2', H-4', H-5'$_B$, H-5", H-6'$_A$, H-6"$_B$), 3.68, 3.78, 3.79 (9H, 3s, OCH_3), 3.98 (1H, dd, *J* 9.3, H-3"), 4.36–4.58 (10H, m, 4 × OC*H*$_2$Ar, H-3', H-4'), 4.65–4.78 (4H, m, 3 × OCH*H*Ar, H-2'), 4.89 (1H, AB, J_{AB} 10.3, OCH*H*Ar), 5.24 (1H, d, *J* 3.4, H-1"), 6.30 (1H, d, *J* 5.9, H-1'), 6.66–6.68 (2H, m, H-3 and H-5 of PMB ring), 6.80–6.68 (4H, m, ArCH), 6.95–6.97 (2H, m, ArCH), 7.04–7.06 (2H, m, ArCH), 7.21–7.37 (21H, m, ArCH), 7.46–7.50 (2H, m, H-4 of Bz ring), 7.85–7.88 (4H, m, H-3

and H-5 of Bz ring), 8.24, 8.55 (2H, 2s, H-2, H-8); *m/z* (FAB^+) 1268 [$(M+H)^+$, 12%], 121 (100).

7.2.17 N^6-benzoyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-2',3",4"-tri-*O*-*p*-methoxybenzyl adenosine (**49**)

47 (290mg, 0.42mmol) And **32** (122mg, 0.21mmol) in dioxane (2.25cm^3) and toluene (0.75cm^3) were stirred with 4Å molecular sieves (approx. 150mg) for 1.75 h, and then dry zinc chloride (34mg, 0.25mmol) and silver perchlorate (104mg, 0.50mmol) were added. The flask was wrapped in foil and stirring was continued for 20 h. Solid $NaHCO_3$ (200mg) and water (2cm^3) were added and the reaction mixture was diluted with ethyl acetate (5cm^3), and was stirred for a further 15 min the mixture was filtered through a Celite pad, and the residue was well washed with ethyl acetate. The filtrate was washed with brine (20cm^3) and saturated aqueous $NaHCO_3$ solution (20cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent ethyl acetate/hexane 7:3) to yield the title compound as a clear oil (70mg, 29%). R_f 0.38 (toluene/ethyl acetate 3:2); (Found: M^+, 1164.505. Calc for $C_{69}H_{72}N_4O_{13}$ $(M+H)^+$: 1164.509); ^1H NMR (270 MHz; $CDCl_3$) δ_H 3.56–3.79 (7H, m, H-2', H-4', H-$5'_A$, H-$5'_B$, H-5", H-$6"_A$, H-$6"_B$), 3.66, 3.78, 3.79 (9H, 3s, 3 × OCH_3), 4.00 (1H, dd, *J* 9.1, H-3"), 4.36–4.60 (10H, m, 4 × OCH_2Ar, H-3', H-4'), 4.72–4.80 (4H, m, 3 × OC*H*HAr, H-2'), 4.91 (1H, AB, J_{AB} 10.4, OC*H*HAr), 5.31 (1H, d, *J* 3.7, H-1"), 6.30 (1H, d, *J* 5.5, H-1'), 6.55–6.58 (2H, m, H-3 and H-5 of PMB ring), 6.80–7.08 (8H, m, ArCH), 7.20–7.32 (21H, m, ArCH), 7.51–7.60 (3H, m, H-3, H-4 and H-5 of Bz ring), 8.03–8.06 (2H, m, H-2 and H-6 of Bz ring), 8.12, 8.76 (2H, 2s, H-2, H-8), 9.21 (1H, br. s, D_2O exch., NH); *m/z* (FAB^+) 1164 [$(M+H)^+$, 10%], 240 (7), 121 (100).

7.2.18 3',4'-Di-*O*-acetyl-N^6-benzoyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-2'-*O*-*p*-methoxybenzyl adenosine (**50**)

133 (182mg, 0.34mmol) and **32** (100mg, 0.17mmol) in dioxane (2.25cm^3) and toluene (0.75cm^3) were stirred with 4Å molecular sieves (approx. 150mg) for 1.75 h, and then dry zinc chloride (56mg, 0.41mmol) and silver perchlorate (171mg, 0.83mmol) were added. The flask was wrapped in foil and stirring was continued for 26 h. Solid $NaHCO_3$ (200mg) and water (2cm^3) were added and the reaction mixture was diluted with ethyl acetate (5cm^3) and stirred for a further 15 min. The mixture was filtered through a Celite pad, and the residue was well washed with ethyl acetate. The filtrate was washed with brine (20cm^3) and saturated aqueous $NaHCO_3$ solution (20cm^3), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent ethyl acetate/hexane 7:3) to yield the title compound as a clear oil (50mg, 29%). R_f 0.57 (toluene/ethyl acetate 1:1); (Found: M^+, 1008.397. Calc for $C_{56}H_{58}N_5O_{13}$ $(M+H)^+$: 1008.403); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.91, 2.01 (6H, 2s, 2 × CH_3CO), 3.35–3.41 (2H, m, H-6"$_A$, H-6"$_B$), 3.61 (1H, dd, *J* 10.0, H-2"), 3.68–3.73 (1H, m, H-5'$_A$, obscured by OCH_3), 3.68 (3H, s, OCH_3), 3.80 (1H, 0.5 ABX, $^2J_{AB}$ 10.5, $^3J_{BX}$ 4.2, H-5'$_B$), 3.87–3.91 (1H, m, H-5"), 4.35–4.66 (8H, m, H-3', H-4', 3 × O*C*H_2Ar), 4.77 (1H, dd, *J* 5.1, H-2'), 5.08 (1H, dd, *J* 9.8, H-4"), 5.30 (1H, d, *J* 3.4, *J* 3.4, H-1"), 5.47 (1H,dd, *J* 9.8, H-3"), 6.24 (1H, d, *J* 5.9, H-1'), 6.59–6.61 (2H, m, H-3 and H-5 of PMB ring), 6.95–6.97 (2H, m, H-2 and H-6 of PMB ring), 7.20–7.34 (15H, m, ArCH), 7.50–7.62 (3H, m, H-3, H-4 and H-5 of Bz ring), 8.03–8.05 (2H, m, H-2 and H-6 of Bz ring), 8.11, 8.73 (2H, 2s, H-2, H-8), 9.71 (1H, br s, D_2O exch., NH); *m/z* (FAB$^+$) 1008 [$(M+H)^+$, 45%], 240 (30), 91 (100).

7.2.19 2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranosyl dimethyl phosphite (**47**)

To a mixture of **46** (2.00g, 3.33mmol) and tetrazole (0.35g, 5.00mmol) in DCM (20cm^3) was added bis(methoxy)(diethylamino)phosphine (0.72cm^3, 4.33mmol). After stirring for 20 min TLC (ethyl acetate/hexane 1:3) indicated complete conversion to

product (R_f 0.66). The reaction mixture was partitioned between diethyl ether (150cm^3) and water (100cm^3). The resulting ethereal layer was washed with brine (100cm^3), dried ($MgSO_4$), filtered and concentrated to give a colourless, mobile oil which was shown by ^{1}H NMR spectroscopy to be a 1:1 anomeric mixture, and which was used for the next step without further purification.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.48–3.74 (11H, m, 2 × $POCH_{3\alpha}$, 2 × $POCH_{3\beta}$, H-2, H-3, H-5, H-6$_A$, H-6$_B$), 3.77, 3.78, 3.78, 3.79 (6H, 4s, 4 × 1.5 OCH_3), 3.94–3.99 (1H, m, H-4), 3.94–3.99 (3H, m, 3 × OCH*H*Ar), 4.70–4.91 (5H, m, 5 × OCH*H*Ar), 4.94 (0.5 H, dd, *J* 8.07, H-1β), 5.54 (0.5H, dd, *J* 3.18, $J_{H\text{-}P}$ 8.55, H-1α), 6.80–6.85 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.05–7.12 (2H, m, H-2 and H-6 of PMB rings), 7.20–7.38 (12H, m, ArCH); α and β subscripts denote signals arising from α and β-anomers respectively; ^{31}P NMR (36 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 141.14 $OP_\beta(OMe)_2$, 142.31 $OP_\alpha(OMe)_2$.

7.2.20 2",5',6"-Tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-2',3",4"-tri-*O*-*p*-methoxybenzyl-N^6-dimethoxytrityl adenosine (**53**)

47 (2.42g, 3.50mmol) And **40** (1.36g, 1.75mmol) in dioxane (18cm^3) and toluene (6cm^3) were stirred with 4Å molecular sieves (approx. 1.80g) for 2 h, and then dry zinc chloride (0.57g, 4.20mmol) and silver perchlorate (1.74g, 8.40mmol) were added. The flask was wrapped in foil and stirring was continued for 7 h. Solid $NaHCO_3$ (1.50g) and water (60cm^3) were added and the reaction mixture was diluted with ethyl acetate (80cm^3), and was stirred for a further 30 min. The mixture was filtered through a Celite pad, and was well washed with ethyl acetate. Water (50cm^3) was added to the filtrate and the resulting aqueous layer was discarded. The organic layer was washed with brine (70cm^3), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7, then 1:1) to yield the title compound as a clear oil (1.26g, 53%).

R_f 0.33 (ethyl acetate/hexane 2:3); $[\alpha]_D^{21}$ +10.0 (*c* 1.5, $CHCl_3$); (Found: C, 71.90; H, 6.18; N, 5.03. Calc for $C_{82}H_{83}N_5O_{14}$: C, 72.28; H, 6.18; N, 5.14%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.43–3.79 (7H, m, H-2", H-4", H-5'$_A$, H-5'$_B$, H-5", H-6"$_A$, H-6"$_B$), 3.69, 3.77, 3.78, 3.79 (15H, 4s, 5 × OCH_3), 3.96 (1H, dd, *J* 9.3, H-3"), 4.34–4.76 (14H, m, 9 × OCH*H*Ar, H-2', H-3', H-4'), 4.87 (1H, AB, J_{AB} 10.3 OCH*H*Ar), 5.22 (1H, d, *J* 3.4, H-1"),

6.21 (1H, d, *J* 4.9, H-1'), 6.65–6.67 (2H, m, ArCH), 6.79–6.86 (10H, m, ArCH), 7.01–7.05 (4H, m, ArCH), 7.21–7.36 (24H, m, ArCH), 7.89, 8.04 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.44, 55.49 (2q, 2 × OCH_3), 68.48 (t, C-6"), 69.52 (t, C-5'), 70.87 (s, DMTr Cq), 71.09 (d, C-5"), 72.36, 72.50 (2t, 2 × OCH_2Ar), 73.01 (d, C-3'), 73.67, 73.75, 74.97, 75.59 (4t, 4 × OCH_2Ar), 77.31 (d, C-4"), 79.65 (d, C-2'), 79.83 (d, C-2"), 81.66 (d, C-3"), 82.32 (d, C-4'), 87.10 (d, C-1'), 96.35 (d, C-1"), 113.38, 113.94, 114.00 (3d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 121.60 (s, C-5), 127.04, 127.90, 127.99, 128.04, 128.12, 128.19, 128.28, 128.52, 128.61, 128.74, 129.07, 129.80, 130.36 (13d ArCH), 129.34, 130.66, 131.30 (3s, 3 × C-1 of PMB ring), 137.75, 137.88, 138.08, 138.39 (4s, C-1 of DMTr *p*-OCH_3 phenyl rings, 3 × C-1 of benzyl rings), 139.07 (d, C-8), 145.76 (s, C-1 of DMTr phenyl ring), 148.83 (s, C-4), 152.51 (d, C-2), 158.49 (s, C-4 of DMTr *p*-OCH_3 phenyl rings), 159.37, 159.46, 159.61 (3s, 3 × C-4 of PMB ring); *m/z* (FAB^+) 1362 [$(M+H)^+$, 8%], 303 (100), 121 (75).

7.2.21 2",5',6"-Tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl adenosine (**54**)

To **53** (1.28g, 0.94mmol) in DCM (63cm^3), was added TFA (7cm^3). The resulting bright orange solution was stirred for 1.75 h before being poured into saturated aqueous $NaHCO_3$ solution (500cm^3). DCM (150cm^3) was added and the now colourless mixture was stirred vigorously for 15 min. The organic layer was collected and the aqueous layer was back-extracted with DCM (2 × 150cm^3). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate/ethanol 14:1) to yield the title compound as a white solid (530mg, 81%).

broad mp 95–115°C (ethanol); R_f 0.28 (ethyl acetate/ethanol 14:1); $[\alpha]_D^{25}$ –2.3 (*c* 2.6, $CHCl_3$); (Found: C, 63.30; H, 5.92; N, 9.80. Calc for $C_{37}H_{41}N_5O_9$, C 63.51; H, 5.91; N, 10.01%); ^{1}H NMR (400 MHz; $(CD_3)_2CO$) δ_H 3.45–3.51 (2H, m, H-2", H-4"), 3.66–3.81 (4H, m, H-5'$_A$, H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.93–3.96 (1H, m, H-5"), 4.04 (1H, dd, *J* 9.0, H-3"), 4.38–4.39 (1H, m, H-4'), 4.50–4.56 (4H, m, 2 × OCH_2Ar), 4.61–4.64 (2H, m, H-3', OH), 4.81–4.87 (5H, m, OCH_2Ar, H-2', 2 × OH), 5.22 (1H, d, *J* 3.9, H-1"), 6.12 (1H, d, *J* 5.4, H-1'), 6.91 (2H, br s, NH_2), 7.20–7.42(15H, m, ArCH), 8.21, 8.25 (2H, 2s, H-2, H-8);

^{13}C NMR (100.4 MHz; $(CD_3)_2CO$) δ_C 70.68 (t, C-6"), 70.74 (t, C-5'), 71.51 (d, C-4"), 73.21 (d, C-5"), 73.75, 73.88, 73.96 (3t, 3 × OCH_2Ar), 74.30 (d, C-3"), 75.11 (d, C-2'), 78.49 (d, C-3'), 80.23 (d, C-2"), 83.05 (d, C-4'), 89.14 (d, C-1'), 99.10 (d, C-1"), 120.35 (s, C-5), 128.11, 128.25, 128.35, 128.46, 129.02, 129.08, 129.15 (7d, ArCH), 139.19, 139.78 (2s, 3 × C-1 of Bn rings), 140.03 (C-8), 150.73 (s, C-4), 153.77 (d, C-2), 157.04 (s, C-6); *m/z* (FAB^+) 700 [$(M+H)^+$, 100%], 91 (99).

7.2.22 Imidazolium triflate (**161**)

Triflic acid (2.00cm^3, 22.6mmol) was added dropwise to imidazole (1.54g, 22.6mmol) in DCM (30cm^3) at 0°C. The cooling bath was removed and the mixture was stirred for 20 min, after which diethyl ether (40cm^3) was added. The resulting precipitated product was then collected by filtration (4.88g, 99%).
mp 196–197°C [lit.[157] 197–198°C]

7.2.23 2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-D-glucopyranosyl adenosine (**55**)

A solution of **54** (100mg, 0.14mmol) in DCM (3cm^3) with bis(benzyloxy)(diisopropylamino)phosphine (0.16cm^3, 0.47mmol) and imidazolium triflate (100mg, 0.46mmol), was stirred for 30 min, after which time TLC (ethyl acetate/hexane 7:3) indicated some starting material remaining; therefore a further 1.0 equivalent each of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate was added. TLC after a further 30 min indicated complete conversion to the tris phosphite (R_f 0.68). Water (1 drop) was added and the solution cooled to -78°C, after which MCPBA (139mg, 0.49mmol) was added. After 10 min 10% aqueous Na_2SO_3 solution (15cm^3) and ethyl acetate (20cm^3) were added and the mixture allowed to warm to room temperature. The resulting organic layer was washed with 15cm^3 each of saturated aqueous $NaHCO_3$ solution and brine, and then dried ($MgSO_4$), filtered and concentrated to a clear oil that was subjected to flash chromatography (eluent

chloroform/acetone 4:1, then 7:3). Concentration of the appropriate fractions gave the title compound as a clear oil (148mg, 70%).

R_f 0.11 (chloroform/acetone 9:1); $[\alpha]_D^{20}$ +11.7 (*c* 3.0, $CHCl_3$); (Found: C, 63.90; H, 5.72; N, 4.56. Calc for $C_{79}H_{80}N_5O_{18}P_3$: C, 64.09; H, 5.45; N, 4.73%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.54–3.69 (4H, m, H-2", H-5'$_A$, H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.83–3.86 (1H, m, H-5"), 4.30 (1H, AB, J_{AB} 11.7, OC*H*HAr), 4.36–4.81 (14H, m, H-4', H-3', H-4", 11 × OC*H*HAr), 4.88–5.07 (8H, m, H-3", 7 × OC*H*HAr), 5.33 (1H, d, *J* 3.4, H-1"), 5.62–5.65 (1H, m, H-2"), 6.09 (2H, br s, NH_2), 6.35 (1H, d, *J* 6.3, H-1'), 6.95–7.40 (45H, m, ArCH), 7.92, 8.24 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 68.51 (t, C-6"), 68.28–70.07 (7t, C-5', 6 × PO*C*H$_2$Ar with C-P coupling), 70.27 (d, C-5'), 71.77, 73.53, 73.76 (3t, 3 × O*C*H$_2$Ar), 73.87, 74.54 (2d, C-3', C-4' with C-P coupling), 77.00 (d, C-2"), 77.40 (d, C-2' with C-P coupling), 78.28 (d, C-3" with C-P coupling), 82.60 (d, C-4'), 85.78 (d, C-1'), 95.60 (d, C-1"), 119.98 (s, C-5), 127.80, 127.93, 128.02, 128.17, 128.26, 128.48, 128.61, 128.65, 128.68, 128.74, 128.79, 129.03, 129.10 (13d, ArCH), 135.16–135.32 (2s, 2 × C-1 of benzylphospho ring with C-P coupling), 135.88, 136.03, 136.38 (3s, 4 × C-1 of benzylphospho ring with C-P coupling), 137.55, 137.81, 138.21 (3s, 3 × C-1 of Bn rings), 139.60 (d, C-8), 150.26 (s, C-4), 153.06 (d, C-2), 155.57 (s, C-6); ^{31}P NMR ($CDCl_3$, 161.7 MHz, ^{1}H decoupled) δ_P -1.31, -2.02, -2.19 (3s); *m/z* (FAB$^+$) 1478 [(M+H)$^+$, 6%], 91 (100).

7.2.24 3-*O*-α-D-Glucopyranosyl adenosine 2',3",4"-trisphosphate (**27**)

A mixture of **55** (59mg, 0.04mmol) and wet 20% palladium hydroxide on carbon (177mg), in methanol (7cm^3), cyclohexane (3.5cm^3) and water (0.5cm^3) was heated at reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed copiously with methanol and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion exchange resin column and eluting with a gradient of 0–100% 150mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20°C) gave the desired product as the free acid (24mg, 92%), which was dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion exchange resin to give, after concentration, the sodium salt.

(Found: M^-, 668.039. Calc for $C_{16}H_{25}N_5O_{18}P_3$ $(M-H)^-$: 668.040); ^1H NMR (400 MHz; D_2O) δ_H 3.60–3.73 (6H, m, H-2", H-5'$_A$, H-5'$_B$, H-5", H-6"$_A$, H-6"$_B$), 3.97 (1H, ddd, *J* 8.9, H-4"), 4.28 (1H, m, H-4'), 4.37 (1H, ddd, *J* 9.3, H-3"), 4.48 (1H, m, H-3'), 5.10–5.14 (2H, m, H-1", H-2'), 6.18 (1H, d, *J* 6.4, H-1'), 8.24, 8.34 (2H, 2s, H-2, H-8); ^{31}P NMR (161.7 MHz; D_2O; ^1H decoupled) δ_P 0.32, 0.87, 1.22 (3s); UV (H_2O) λ_{max} 259nm, ε 15 400, pH 7.5; *m/z* (FAB^-) 668 [$(M-H)^-$, 100%], 266 (34), 113 (44).

7.3 Synthesis of ribophostin

7.3.1 Allyl D-glucopyranoside (64)

A solution of D-glucose (100.23g, 0.60mol) in allyl alcohol (500cm^3) was heated at reflux with Dowex 50X2-100 (25.00g) for 3 h. The resulting orange solution was cooled, filtered and the residue washed well with ethanol (100cm^3). The filtrate and washings were combined and concentrated. The syrupy orange residue was subjected to flash chromatography (eluent ethyl acetate/methanol 9:1). Fractions containing the title compound were combined and concentrated to yield an anomeric mixture as a yellow oil that became a white solid on standing (86.54g, 71%), which was shown by ^{1}H NMR (270 MHz; D_2O) to be a ca. 2:1 α:β-anomeric mixture (δ_H 4.48, J 7.9, H-1$_\beta$; δ_H 4.94, J 3.7, H-1$_\alpha$).

Crystals of the α-anomer were isolated from three fractions.

mp 101–102°C (ethyl acetate/methanol), [lit.[214] 100.5–101.5°C].

7.3.2 Allyl 2,6-di-O-benzyl-α-D-glucopyranoside (65)

The 7:3 anomeric mixture of **64** (43.57g, 0.20mol) was heated under reflux in dry toluene (1000cm^3), with dibutyl tin oxide (127.00g, 0.50mol). Continuous azeotropic removal of water was carried out by means of a Dean-Stark trap over 3 h. The solution was cooled and concentrated to give a white residue, which was dried *in vacuo* for 5 h. Benzyl bromide (350cm^3) was added to the powdered residue and the mixture was stirred for 3 days at 80–90°C under an atmosphere of nitrogen. The resulting yellow solution was cooled and diluted with diethyl ether (600cm^3). Saturated aqueous $NaHCO_3$ solution (500cm^3) was added, and the mixture stirred vigorously for 30 min. The resulting suspension was filtered through Celite and the organic layer was separated, dried ($MgSO_4$), filtered and concentrated to give a dark yellow oil, which was subjected to flash chromatography (eluent hexane/ethyl acetate 8:2 to remove benzyl bromide, then 1:1, then 2.5:7.5). Fractions containing a mixture of benzylated products were combined

(13.18g R_f 0.30-0.51 ethyl acetate) and crystallisation from diisopropyl ether gave the title compound (7.90g, 10%).

R_f 0.22 (ethyl acetate/hexane 3:2); mp 73–75°C [lit.[170]. 74–77°C] (diisopropyl ether); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 2.68–2.72 (2H, m, H-6_A, H-6_B), 3.39 (1H, dd, *J* 3.5, 9.7, H-2), 3.61–3.78 (4H, m, H-4, H-5, 2 × OH), 3.92–3.97 (2H, m, H-3, C*H*HCH=CH_2), 4.11–4.18 (1H, m, CH*H*CH=CH_2), 4.55 (1H, AB, J_{AB} 12.1, OC*H*HAr), 4.59–4.66 (3H, m, 3 × OC*H*HAr), 4.83 (1H, d, *J* 3.5, H-1), 5.20 (1H, m, ^3J 10.4, CH_2CH=C$H_{cis}CH_{trans}$), 5.31 (1H, m, ^3J 17.2, CH_2CH=$CH_{cis}CH_{trans}$), 5.86–5.91 (1H, m, CH_2CH=CH_2), 7.25–7.34 (10H, m, ArCH).

7.3.3 Allyl 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (**66**)

Sodium hydride (1.98g of a 60%$^w/_w$ dispersion in mineral oil, 0.05mol), and *p*-methoxybenzyl chloride (4.10cm^3, 0.03mol) were added sequentially to a stirring solution of **65** stirring in dry DMF (50cm^3). Stirring was continued for 3 h when TLC (ethyl acetate/hexane 3:2) showed consumption of starting material (R_f 0.22) to give a product (R_f 0.71). Methanol (20cm^3) was added and stirring continued for 15min. The solvents were evaporated and the residue was dissolved in chloroform (600cm^3). This organic solution was washed with water (3 × 100cm^3), dried ($MgSO_4$), filtered and concentrated. Flash chromatography of the residual oil (eluent hexane/ethyl acetate 19:1) gave the title compound as a pale yellow oil (7.91g, 88 %).

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.55 (1H, dd, *J* 3.5, 9.5, H-2), 3.59–3.63 (2H, m, H-4, H-6_A), 3.68–3.75 (2H, m, H-5, H-6_B), 3.78, 3.80 (6H, 2s, 2 × OCH_3), 3.94–4.16 (2H, m, H-3, CH_2CH=CH_2), 4.36–4.93 (9H, m, 4 × OCH_2Ar, H-1), 5.20 (1H, dd, ^3J 10.3, ^2J 0.9, CH_2CH=C$H_{cis}H_{trans}$), 5.29 (1H, dd, ^3J 17.2, ^2J 1.0, CH_2CH=$CH_{cis}H_{trans}$), 5.92 (1H, m, CH_2CH=CH_2), 6.78-6.88 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.04 (2H, d, *J* 8.4, H-2 and H-6 of PMB rings), 7.25-7.34 (12H, m, ArCH).

7.3.4 2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose (**46**)

A solution of **66** (7.90g, 0.01mol) and freshly sublimed potassium *t*-butoxide (6.9g, 0.06mol), in dry DMSO (100cm^3) was stirred at 50°C in for 3 h. TLC (hexane/ethyl acetate 4:1) indicated consumption of starting material (R_f 0.33) to give a product (R_f 0.37). After cooling, water (80cm^3) was added to the dark brown solution, and the system extracted with diethyl ether (3 × 275cm^3). The combined organic extracts were washed with saturated aqueous KCl solution (3 × 200cm^3), dried ($MgSO_4$), filtered and concentrated to a pale yellow oil. Crude (*cis*-prop-1-enyl) 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-glucopyranoside was then dissolved in acetone (160cm^3), and the solution was heated to 50°C. Aqueous 1M HCl (24cm^3) was added and stirring continued for 50 min, when TLC (ethyl acetate/hexane 1:4) indicated consumption of starting material (R_f 0.37) to give a product (R_f 0.03). Solid $NaCO_3$ (10g) was added and stirring continued as the suspension was allowed to cool to rt. The solvents were evaporated and the residue was extracted with diethyl ether (3 × 250cm^3). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ solution (200cm^3) and water (200cm^3), dried ($MgSO_4$), filtered and concentrated. Recrystallisation from a minimum of diethyl ether gave the title compound (5.66g, 76%; from allyl D-glucopyranoside).

mp 117–120°C [lit.[170] 120–131°C] (diethyl ether); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 1.67 (0.5H, s, D_2O exch., OH_β), 3.18 (0.5H, d, *J* 2.6, D_2O exch., OH_α), 3.37 (0.5H, d, *J* 7.6, 9.9, H-2$_\beta$), 3.50–3.80 (4.5H, m, H-2$_\alpha$, H-3$_\alpha$ or H-3$_\beta$, H-5$_\alpha$ or H-5$_\beta$, H-4, H-6$_A$, H-6$_B$), 3.78, 3.79, 3.80 (6H, 3s, 2 × OCH_3), 3.94 (0.5H, dd, *J* 9.2, H-3$_\alpha$ or H-3$_\beta$), 4.01 (0.5H, ddd overlapping with t at 3.94 ppm, H-5$_\alpha$ or H-5$_\beta$), 4.39–4.93 (8.5H, m, H-1$_\beta$, 4 × OCH_2Ar), 5.21 (0.5H, dd, *J* 2.6, simplifies to d on D_2O exch., H-1$_\alpha$), 6.78–6.87 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.03–7.07 (2H, m, H-2 and H-6 of PMB ring), 7.21–7.34 (10H, m, ArCH).

7.3.5 2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-*β*-D-glucopyranosyl trichloroacetimidate (**67a**)

To a solution of **46** (1.00g, 1.67mmol) in freshly distilled dry DCM (10cm^3) was added freshly flame dried K_2CO_3 (1.00g, 7.25mmol), followed by freshly distilled trichloroacetonitrile (1.00cm^3). The mixture was left to stir at room temperature under an atmosphere of nitrogen for 140 min, after which time TLC (chloroform/acetone 30:1) indicated a small amount of starting material (R_f 0.14), a major product (R_f 0.47) and a minor product (R_f 0.58). The reaction mixture was filtered through a pad of celite and concentrated to a clear oil. This oil was purified by flash chromatography (eluent chloroform/acetone 50:1), to give the title compound (0.59g, 48%), and its *α*-anomer (0.25g, 20%). The title compound was crystallised from a minimum amount of diethyl ether/petroleum ether 1:1, overnight at -4°C.

Analyses for *β*-anomer (**67a**): mp 80–81°C (petroleum ether/ether); $[\alpha]_D$ +16.45 (*c* 4.7, $CHCl_3$); (Found: C, 61.40; H, 5.41; N 1.86. Calc for $C_{38}H_{40}N_1O_8Cl_3$: C, 61.26; H, 5.41; N, 1.88%); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.56–3.73 (6H, m, H-2, H-3, H-4, H-5, H-6$_A$, H-6$_B$), 3.79, 3.80 (6H, 2s, 2 × OCH_3), 4.47–4.78 (6H, m, 3 × O*C*H_2Ar), 4.82 (1H, AB, J_{AB} 10.4, OCH*H*Ar), 4.93 (1H, AB, J_{AB} 11.0, OCH*H*Ar), 5.78 (1H, d, *J* 8.3, H-1), 6.79–6.85 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.09 (2H, m, H-2 and H-6 of PMB ring), 7.20–7.31 (12H, m, ArCH), 8.69 (1H, s, C=NH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.27 (q, 2 × OCH_3), 68.21 (t, C-6), 73.35, 74.60, 74.91, 75.32 (4t, 4 × O*C*H_2Ar), 75.90, 77.03, 81.00, 84.29 (4d, C-2, C-3, C-4, C-5), 98.35 (d, C-1), 113.79, (2d, C-3 and C-5 of PMB rings), 127.61, 127.76, 127.89, 127.93, 128.36, 128.47, 129.46, 129.64, (7d, ArCH), 130.19, 130.63 (2s, 2 × C-1 of PMB rings), 138.02, 138.11 (2s, 2 × C-1 of Bn rings), 159.20 (2s, 2 × C-4 of PMB rings) 161.23 (C=NH); *m/z* (FAB$^-$) 744 [(M-H)$^-$, 34%], 311 (73),188 (100).

Analyses for *α*-anomer (**67b**): $[\alpha]_D$ +13.64 (*c* 1.3, $CHCl_3$); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 3.65 (1H, 0.5 ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 1.9, H-6$_A$), 3.71–3.80 (3H, m, H-2, H-4, H-6$_B$), 3.78, 3.79 (6H, 2s, 2 × OCH_3), 3.94–4.06 (2H, m, H-3, H-5), 4.45, 4.77 (2H, AB, *J* 10.3, O*C*H_2Ar), 4.47, 4.60 (2H, AB, *J* 12.1, O*C*H_2Ar), 4.69, 4.74 (2H, AB, *J* 11.7, O*C*H_2Ar), 4.76, 4.87 (2H, AB, *J* 10.4, O*C*H_2Ar), 6.51 (1H, d, *J* 3.3, H-1), 6.79–6.85 (4H, m, 2 × H-

3 and H-5 of PMB rings), 7.05–7.08 (2H, m, H-2 and H-6 of PMB ring), 7.23–7.32 (12H, m, ArCH), 8.57 (1H, s, C=NH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.27 (q, 2 × OCH_3), 68.03 (t, C-6), 72.88, 73.46, 74.94, 75.31 (4t, 4 × OCH_2Ar), 73.17, 76.50, 79.43, 81.07 (4d, C-2, C-3, C-4, C-5), 94.42 (d, C-1), 113.77, 113.83 (2d, C-3 and C-5 of PMB rings), 127.59, 127.69, 127.94, 128.36, 129.68, 129.74 (6d, ArCH), 130.28, 130.85 (2s, 2 × C-1 of PMB rings), 137.91, 138.04 (2s, 2 × C-1 of Bn rings), 159,12, 159.31 (2s, 2 × C-4 of PMB rings), 161.32 (s, C=NH); *m/z* (FAB$^-$) 744 [(M-H)$^-$, 1%], 121 (100).

7.3.6 Methyl 2',5,6'-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-2,3',4'-tri-*O*-*p*-methoxybenzyl-β-D-ribofuranoside (**72a**)

A mixture of **67a** (188mg, 0.25mmol), and methyl 5-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-ribofuranoside (86mg, 0.23mmol) was stirred in dry diethyl ether (2.5cm^3) at room temperature with 4Å molecular sieves (90mg) for 30 min, whereupon trimethylsilyl triflate (0.01cm^3 of a 0.2M solution in diethyl ether, 0.002mmol) was added. After 5 min TLC (chloroform/acetone 30:1) showed formation of a product (R_f 0.26) from the imidate (R_f 0.32), and the glycosyl acceptor (R_f 0.23). The reaction mixture was quenched with triethylamine (6 drops), and concentrated. The resulting clear oil was subjected to flash chromatography (eluent chloroform/acetone 30:1), and again (eluent chloroform/acetone 35:1), to give an inseparable mixture of the title compound, and its β-D-glucopyranosyl isomer, in a 4:1 ratio (120mg, 54%) calculated from ^{1}H NMR integral ratios.

(Found: C, 71.5; H, 6.72. Calc for $C_{57}H_{64}O_{13}$: C, 71.53; H, 6.74%); Selected ^{1}H NMR data for α-coupled product: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 4.93 (1H, d, *J* 2.0, H-1), 5.09 (1H, d, *J* 3.4, H-1'); *m/z* (FAB$^-$) 1109 [(M+153)$^-$, 100%].

7.3.7 Methyl 2',5,6'-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-β-D-ribofuranoside (**73a**)

72a (379mg, 0.40mmol) Was stirred with DCM ($12cm^3$) and water ($1cm^3$), for 20 min, when DDQ (551mg, 2.38mmol) was added. After 60 min TLC (chloroform/acetone 30:1) showed consumption of starting material R_f 0.26. The reaction mixture was diluted with DCM ($60cm^3$), and the organic layer washed with 10% aqueous Na_2SO_3 solution ($3 \times 50cm^3$), followed by $50cm^3$ each of saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$) filtered and concentrated to a clear oil, which was subjected to flash chromatography (eluent ethyl acetate/hexane 7:3), to give methyl 2',5,6'-tri-*O*-benzyl-3-*O*-β-D-glucopyranosyl-β-D-ribofuranoside (**73b**) (27mg, 11%).

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 2.86, 2.30, 3.06 (3H, 3 × br. s, 3 × OH), 3.23–3.27 (1H, m, H-2'), 3.33 (3H, s, OCH_3), 3.40–3.49 (3H, m, H-3', H-4', H-5'), 3.56 (1H, 0.5 ABX, $^2J_{AB}$ 10.5, $^3J_{AX}$ 5.6, H-5_A), 3.59–3.63 (2H, m, H-5_B, H-$6'_A$), 3.70 (1H, 0.5 ABX, $^2J_{AB}$ 10.5, $^3J_{AX}$ 3.2, H-$6'_B$),4.11 (1H, d, *J* 4.4, H-2), 4.18 (1H, dd, *J* 4.4, 6.8, H-3), 4.28–4.33 (1H, m, H-4), 4.45 (1H, d, *J* 7.8, H-1'), 4.50–4.54 (4H, m, 2 × O*CH*$_2$Ar), 4.58, 4.81 (2H, AB, J_{AB} 11.5, O*CH*$_2$Ar), 4.90 (1H, s, H-1), 7.23–7.35 (15H, m, ArCH).

Further elution gave the title compound (**73a**) (135mg, 57%). Crystallisation occurred spontaneously on standing.

mp 103–105°C; $[\alpha]_D$ +28.26 (*c* 3.7, $CHCl_3$); (Found: M^+, 596.259. Calc for $C_{33}H_{40}O_{10}$ M^+: 596.262); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.69 (1 H, br. s, D_2O exch., OH), 2.74, 2.87 (2H, 2 br s, D_2O exch., OH), 3.32 (3H, s, OCH_3), 3.38 (1H, dd, *J* 9.8, 3.4, H-2'), 3.45–3.57 (5H, m, H-4', H-5_A, H-5_B, H-$6'_A$, H-$6'_B$), 3.74 (1H, ddd, *J* 9.8, 3.9, H-5'), 3.92 (1H, dd, *J* 9.3, 9.3, H-3'), 4.01 (1H, m, H-3), 4.22 (2H, m, H-2, H-4), 4.44, 4.52 (2H, AB, *J* 12.2, O*CH*$_2$Ar), 4.51 (2H, s, O*CH*$_2$Ar), 4.69, 4.74 (2H, AB, *J* 11.7, O*CH*$_2$Ar), 4.69 (1H, d, *J* 3.4, H-1'), 4.88 (1H, s, H-1), 7.23–7.36 (15H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.03 (q, OCH_3), 69.00 (t, C-5 or C-6'), 70.74 (d, C-4'), 70.84 (d, C-5'), 71.69 (t, C-5 or C-6'), 73.24 (d, C-3'), 73.28 (C-3), 73.32, 73.55, 74.14 (3t, 3 ×O*CH*$_2$Ar), 78.35 (d, C-2'), 79.11 (d, C-2 or C-4), 80.27 (d, C-2 or C-4), 97.79 (d, C-1'), 108.34 (d, C-1), 127.57, 127.63, 127.69, 127.74, 128.33, 128.42, 128.51, 128.75 (8d, ArCH),

137.18, 137.86, 138.06 (3s, 3 × C-1 of Bn rings); *m/z* (FAB^+) 597 [$(M+1)^+$, 12%], 565 (48), 343 (3), 255 (2).

7.3.8 Methyl-2',5,6'-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-2,3',4'-tris-(dibenzyloxyphosphoryl)-β-D-ribofuranoside (**74**)

A mixture of bis(benzyloxy)(diisopropylamino)phosphine (372mg, 1.08mmol), dry DCM (3mL) and tetrazole (113mg, 1.62mmol) was stirred at room temperature for 30 min, after which time a solution of **73a** (107mg, 0.18mmol) in dry DCM ($2cm^3$) was added and stirring was continued for a further 30 min. TLC (ethyl acetate/hexane 7:3) indicated complete conversion of starting material (R_f 0.14) to a product (R_f 0.49) and ^{31}P NMR spectroscopy showed phosphite triester signals. The system was cooled to -78°C, MCPBA (432mg, 2.15mmol) was added and the system warmed to room temperature. After 10 min, TLC showed conversion of the trisphosphite to a new product (R_f 0.78). The mixture was extracted with DCM ($100cm^3$) and the organic extract was washed with 10% aqueous Na_2SO_3 solution ($50cm^3$), saturated aqueous $NaHCO_3$ solution (2 × $50cm^3$), and brine ($50cm^3$). The organic solution was dried ($MgSO_4$), filtered and concentrated to give a white solid. Flash chromatography (eluent $CHCl_3$/acetone 20:1 then 10:1, then 4:1) to gave the title compound as a colourless oil (220mg, 89%).

$[\alpha]_D$ +34.70 (*c* 8.07, $CHCl_3$); (Found: M^+, 1377.451. Calc for $C_{75}H_{80}O_{19}P_3$ M^+: 1377.450); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.25 (3H, s, OCH_3), 3.52 (1H, 0.5 ABX, $^2J_{AB}$ 10.5, $^3J_{AX}$ 5.1, H-5_A), 3.55–3.60 (2H, m, H-2', H-5_B), 3.65 (2H, ABX, $^2J_{AB}$ 10.4, $^3J_{AX}$ = $^3J_{BX}$ 3.9, H-$6'_A$, H-$6'_B$), 3.82–3.84 (1H, m, H-5'), 4.28–4.31 (2H, AB, J_{AB} 11.7, OCH*H*Ar overlapping with H-4), 4.34–4.53 (5H, m, 4 × OCH*H*Ar, H-3), 4.60 (1H, dd, *J* 9.5, 18.8, H-4'), 4.67–4.77 (3H, m, 2 × OCH*H*Ar, H-2), 4.80–5.03 (13H, m, H-1, H-3', 11 × OCH*H*Ar), 5.09 (1H, d, *J* 3.4, H-1'), 7.01–7.35 (45H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.19 (q, OCH_3), 67.94 (t, C-5 or C-6'), 69.01 69.29, 69.41, 69.56, 69.71, 69.78 (6t, 6 × POCH_2Ar), 70.57 (t, C-5 or C-6'), 71.91, 73.19 (2t, 3 × OCH_2Ar), 73.95 and 74.30 (with C-P coupling), 76.86, 77.08 and 78.18 (with C-P coupling), 79.75 (6d, C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 95.01 (d, C-1'), 105.85 (d, C-1), 127.39–128.95

(45d, ArCH), 135.53–136.19 (6s, 6 × C-1 of benzylphospho rings, with C-P coupling), 137.62, 138.06, 138.11 (3s, 3 × C-1 of Bn rings); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P -2.25, -1.94, -1.22 (3s); *m/z* (FAB^+) 1377 (M^+, 4%), 91 (100).

7.3.9 Methyl 3-O-α-D-glucopyranosyl-β-D-ribofuranoside 2,3',4'-trisphosphate (**62**)

10% Palladium on activated charcoal (200mg), was added to a solution of **74** in methanol (40cm^3) and water (10cm^3). This mixture was shaken under 40 psi pressure in an atmosphere of hydrogen for 18 h, after which it was filtered through Celite. The filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300cm^3) and purified by ion-exchange chromatography on Q Sepharose fast flow resin, eluting with a gradient of triethylammonium bicarbonate buffer (0–1M), pH 7.5. The triethylammonium salt of the title compound eluted over 800-850 mmol dm^{-3} buffer. After concentration of the appropriate fractions **62** was found to be insoluble in water. Therefore **62** was converted into its potassium salt with 0.1M aqueous potassium hydroxide solution (2cm^3), and subsequently quantified by the Briggs total phosphate assay (60μmol, 78%).

$[\alpha]_D^{20}$ +79.1 (*c* 1.70 calc. for free acid, MeOH); (Found: M^-, 565.012. Calc for $C_{12}H_{24}O_{19}P_3$ $(M-H)^-$: 565.012); ^{1}H NMR (400 MHz; CD_3OD) δ_H 3.35 (3H, s, OCH_3), 3.54 (1 H, dd, *J* 6.4, 11.9, H-5$_A$), 3.62 (1H, dd, *J* 3.8, 9.6, H-2'), 3.66 (1H, br s, OH), 3.69–3.73 (3H, m, H-5$_B$, H-5', H-6'$_A$), 3.93 (1H, dd, *J* 3.5, 13.0, H-6'$_B$), 4.04–4.11 (2H, m, H-4, H-4'), 4.44 (1H, dd, *J* 4.3, 7.3, H-3), 4.45 (1H, dd, *J* 9.0, 18.2, H-3'), 4.58 (1H, dd, *J* 4.3, 9.5, H-2), 4.94 (1H, s, H-1), 5.03 (2H, br s, 2 × OH), 5.13 (1H, d, *J* 3.7, H-1'); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.18 (q, OCH_3), 61.98, 64.87 (2t, C-5, C-6'), 73.32, 73.52, 73.78, 76.29, 76.49 (with C-P coupling), 78.83 (6d, C-2, C-3, C-2'–C-5'), 82.71 (d, C-4), 98.93 (d, C-1'), 108.96 (dd, $^3J_{CP}$ 3.7, C-1) and triethylammonium signals; ^{31}P NMR (161.7 MHz; CD_3OD; ^{1}H decoupled) δ_P -0.38, 1.05, 1.10 (3s); *m/z* (FAB^-) 565 ($[M-H]^-$ 100%).

7.4 Synthesis of furanophostin

7.4.1 [(3*S*,4*R*)-3-*p*-methoxybenzyloxy tetrahydrofuran-4-yl] 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (**76**)

A mixture of **47** (1.85g, 2.68mmol) and (+)-(3*R*,4*S*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol (0.30g, 1.34mmol) was stirred together with 4Å molecular sieves (1.50g) in of toluene (5cm^3) and dioxane (15cm^3) for 2h under an atmosphere of nitrogen. Zinc chloride (0.44g) and silver perchlorate (1.33g) were then added and the flask was wrapped in foil. After stirring for 2 h TLC (ethyl acetate/toluene 1 : 4) indicated the formation of one major product (R_f 0.44). The foil was removed and water (25 cm^3) added, the resulting pale pink suspension was filtered through Celite and the residue was washed thoroughly with ethyl acetate. The filtrate and washings were transferred to a separating funnel with ethyl acetate (150 cm^3) and water (100 cm^3). The organic layer was collected, washed with brine (100 cm^3), dried ($MgSO_4$), filtered and concentrated. The resulting clear residue was subjected to flash chromatography (eluent ethyl acetate/toluene 1:9) yielding the title compound as a clear oil which solidified on standing (0.82g, 76%).

mp 80–83°C (diethyl ether/hexane); $[\alpha]_D^{20}$ + 19.0 (*c* 0.2, $CHCl_3$); (Found: C, 71.30; H, 6.75. Calc. for $C_{48}H_{54}O_{11}$: C, 71.45; H, 6.74%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.54–3.62 (3H, m, H-2, H-4, H-6$_A$), 3.68–3.87 (13H, m, 3 × OCH_3, H-2'$_A$, H-5, H-5'$_A$, H-6$_B$), 3.93–4.02 (3H, m, H-2'$_B$, H-3, H-5'$_B$), 4.07 (1H, dd, *J* 10.5, 5.6, H-3'), 4.20 (1H, dd, *J* 10.5, 5.6, H-4'), 4.36–4.89 (10H, m, 5 × O*CH*$_2$Ar), 5.18 (1H, d, *J* 3.4, H-1), 6.77–6.83 (6H, m, 3 × H-3 and H-5 of PMB rings), 7.02–7.05 (2 H, m, H-2 and H-6 of PMB rings), 7.21–7.32 (14H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.17, 55.18 (2q, 2 × OCH_3), 68.33 (t, C-6), 70.23 (t, C-2', or C-5'), 70.45 (t, C-2', or C-5'), 70.67 (d, C-5), 72.01, 72.30, 73.38, 74.64, 75.24 (5t, O*CH*$_2$Ar), 75.94 (d, C-4'), 77.17 (d, C-4), 77.33 (d, C-3'), 79.74 (d, C-2), 81.33 (d, C-3), 96.90 (d, C-1), 113.69 (3d, C-3 and C-5 of PMB rings), 127.51, 127.64, 127.73, 127.84, 128.23, 128.32, 129.47, 129.54 (8d, ArCH), 130.09, 130.36, 130.99 (3s, 3 × C-1 of PMB rings), 137.83, 138.34 (2s, 2 × C-1 of Bn

rings), 159.06, 159.13, 159.17 (3d, 3 × C-4 of PMB rings); *m/z* (FAB^+) 829 [$(M+Na)^+$, 3%]; 121 (100).

7.4.2 [(3*S*,4*R*)-3-Hydroxytetrahydrofuran-4-yl] 2,6-di-*O*-benzyl α-D-glucopyranoside (**77**)

TFA ($2cm^3$) was added to a solution of **76** (500mg, 0.62mmol) in DCM ($18cm^3$) and the resulting purple solution was stirred at rt. for 10 min, then poured slowly into saturated aqueous $NaHCO_3$ solution ($300cm^3$). The aqueous layer was extracted with DCM (3 × $100cm^3$) and the combined organic layer was dried ($MgSO_4$), filtered and concentrated to a yellow oil that was subjected to flash chromatography (eluent ethyl acetate/hexane 7 : 3), to give a white solid (234mg, 84%).

mp 83–85°C (diethyl ether); $[\alpha]_D^{20}$ +70.0 (*c* 0.4, $CHCl_3$); (Found: C 64.40; H 6.78. Calc for $C_{24}H_{30}O_8$: C 64.56; H 6.77); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.37 (1H, dd, *J* 9.5, 3.7, H-2), 3.47 (1H, ddd, *J* 9.3, 3.9, H-4), 3.63 (2H, d, *J* 3.9, H-6_A, H-6_B), 3.69 (1H, dd, *J* 9.8, 4.4, H-$2'_A$), 3.74–3.79 (2H, m, H-5, H-$5'_A$), 3.82–3.90 (2H, m, H-$2'_B$, H-$5'_B$), 3.93 (1H, ddd, *J* 9.0, 3.9, H-3), 3.99 (1H, ddd, *J* 5.4, H-4'), 4.07–4.11 (2H, m, D_2O exch., 3'-OH, 4-OH), 4.21 (1H, dddd, *J* 10.3, 5.4, 5.4, H-3'), 4.44 (1H, br s, D_2O exch., 3-OH), 4.49, 4.53, (2H, AB, J_{AB} 12.2, O*C*H_2Ar), 4.63, 4.76 (2H, AB, J_{AB} 11.7, O*C*H_2Ar), 4.75 (1H, s, H-1), 7.22–7.36 (10H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 68.94 (t, C-6), 70.23 (d, C-4), 70.31 (t, C-2'), 70.60 (d, C-3'), 71.08 (d, C-5), 72.48 (t, C-5'), 72.92 (d, C-3), 73.32, 73.93 (2t, 2 × O*C*H_2Ar), 78.81 (d, C-2), 78.90 (d, C-4'), 98.4 (d, C-1), 127.42, 127.46, 128.19, 128.33, 128.48 (5d, ArCH), 137.11, 137.75 (2s, 2 × C-1 of Bn rings); *m/z* (FAB^+) 829 [$(M+Na)^+$, 3%]; 121 (100).

7.4.3 [(3*S*,4*R*)-3-Dibenzyloxyphosphoryloxytetrahydrofuran-4-yl] 2,6-di-*O*-benzyl-3,4-bis-*O*-(dibenzyloxyphophoryl) α-D-glucopyranoside (**78**)

A mixture of bis(benzyloxy)(diisopropylamino)phosphine (325mg, 0.94mmol), dry DCM ($5cm^3$) and tetrazole (99mg, 1.41mmol) was stirred at room temperature for 30

min after which the triol **77** (70mg, 0.16mmol) was added and the mixture stirred for a further 30 min. The mixture was then cooled to -78°C and MCPBA (406mg) added, the cooling bath was removed and the mixture was allowed to warm to room temperature. TLC (ethyl acetate) after 15 min indicated conversion of the trisphosphite triester to product (R_f 0.28). The reaction mixture was diluted with DCM (50cm^3) and washed with 25cm^3 each of 10% aqueous Na_2SO_3 solution, saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated to give an off white solid that was subjected to flash chromatography (eluent ethyl acetate/hexane 3:2). Concentration of appropriate fractions gave the title compound as a clear oil (188mg, 98%).

$[\alpha]_D^{25}$ +30.0 (*c* 1.5, $CHCl_3$); (Found: $(M+1)^+$, 1227.382. Calc for $C_{66}H_{70}O_{17}P_3$ $(M+1)^+$: 1227.382); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.56 (1H, dd, *J* 9.8, 3.7, H-2), 3.69–3.75 (2H, m, H-6_A, H-6_B), 3.77–3.82 (2H, m, H-5, H-5'$_A$), 3.88 (2H, d, *J* 5.2, H-2'$_A$, H-2'$_B$), 3.92 (1H, dd, *J* 5.8, 9.2, H-5'$_B$), 4.08 (1H, dd, *J* 10.4, 5.2, H-4'), 4.36, 4.48 (2H, AB, J_{AB} 12.1, OC*H*$_2$Ar), 4.50–4.63 (3H, m, H-4, OC*H*$_2$Ar), 4.73 (1H, AB, J_{AB} 11.9, $J_{H\text{-}P}$ 8.2, POC*H*$_2$Ar), 4.80–5.05 (14H, m, 5.5 × OC*H*$_2$Ar, H-1, H-3, H-3'), 7.03–7.35 (40H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 68.11 (t, C-6), 68.95–69.76 (6t, POC*H*$_2$Ar with C-P coupling, and d, C-5'), 70.00 (t, C-2'), 72.15 (t, OC*H*$_2$Ar), 73.21 (t, OC*H*$_2$Ar), 74.37 (d, C-4 with C-P coupling), 74.70 (d, C-4' with C-P coupling), 75.42 (d, C-3' with C-P coupling), 77.22 (d, C-2), 78.06 (d, C-3 with C-P coupling), 95.76 (d, C-1), 127.53, 127.62, 127.67, 127.79, 127.95, 128.01, 128.19, 128.24, 128.35, 128.44, 128.54 (11d, ArCH), 135.50, 135.60, 135.74, 135.81, 136.07, 136.13 (6s, 6 × C-1 of benzylphospho rings), 137.77, 137.95 (2s, 2 × C-1 of Bn rings); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P -2.25, -1.94, -1.10 (3s); *m/z* (FAB$^+$) 1227 [$(M+1)^+$, 9%], 91 (100).

7.4.4 [(3S,4R)-3-hydroxytetrahydrofuran-4-yl] α-D-glucopyranoside 3,3',4-trisphosphate (79)

Moist palladium on carbon 10% (200mg) was added to a solution of **78** (75mg, 61μmol) in methanol (50cm^3) and water (10cm^3). This mixture was shaken in an atmosphere of hydrogen at 50psi for 25 h. The catalyst was removed by filtration through a membrane filter, and the filtrate was concentrated to a clear residue. The crude

product was purified by ion exchange chromatography using Q Sepharose fast flow resin, eluting with a gradient of 1M triethylammonium bicarbonate buffer (0–1M) pH 8.0. The triethylammonium salt of the title compound eluted between 560 and 620 mmol buffer (57 μmol, 93%).

$[\alpha]_D^{21}$ +74.8 (*c* 0.6 calc. for free acid, MeOH); (Found $(M-H)^-$ 504.9890. Calc for $C_{10}H_{20}O_{17}P_3$ $(M-H)^-$ 504.9913); ^1H NMR (400 MHz; D_2O) δ_H 3.74 (1H, dd, *J* 9.0, 3.2, H-2), 3.78–3.94 (5H, m, H-2'$_A$, H-5, H-5'$_A$, H-6$_A$, H-6$_B$), 4.01–4.09 (3H, m, H-2'$_B$, H-4, H-5'$_B$), 4.43–4.50 (2H, m, H-3, H-4'), 4.80–4.89 (1H, m, H-3'), 5.25 (1H, d, *J* 2.93, H-1); ^{31}P NMR (161.7 MHz; CD_3OD; ^1H coupled) δ_P 0.04 (d, J_{HP} 7.3), 0.82 (d, J_{HP} 7.6), 1.08 (d, J_{HP} 9.8); *m/z* (FAB^-) 505[$(M-H)^-$, 100%].

7.5 Synthesis of mannophostin

7.5.1 2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-mannopyranosyl dimethyl phosphite (**105**)

To a mixture of 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-mannopyranose (1.29g, 2.15mmol) and tetrazole (0.23g, 3.23mmol) in DCM (15cm^3) was added bis(methoxy)(diethylamino)phosphine (0.46cm^3, 2.80mmol). After stirring for 20 min TLC (ethyl acetate/toluene 1:4) indicated complete conversion to product (R_f 0.69). The reaction mixture was partitioned between diethyl ether (80cm^3) and water (60cm^3). The resulting ethereal layer was washed with brine (60cm^3), dried ($MgSO_4$), filtered and concentrated to give a clear runny oil that was used without further purification.
^{1}H NMR (400 MHz; $CDCl_3$) α anomer; δ_H 3.39–3.44 (6H, m, $OP(OMe)_2$ with C-P coupling), 3.65–3.71 (2H, m, H-2, H-6$_A$), 3.76–3.80 (1H, m, H-6$_B$ overlapping with 2 × OCH_3), 3.76, 3.78 (6H, 2s, 2 × OCH_3), 3.90–4.03 (3H, m, H-3, H-4, H-5), 4.44–4.82 (8H, m, 4 × OCH_2Ar), 5.52 (1H, dd, *J* 1.8, $J_{H\text{-}P}$ 8.2, H-1), 6.78–6.86 (4H, m, C-3 and C-5 of PMB ring), 7.08–7.11 (2H, m, C-2 and C-6 of PMB ring), 7.21–7.39 (12H, m, ArCH); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 140.83 $OP_\beta(OMe)_2$, 141.14 $OP_\alpha(OMe)_2$.

7.5.2 2",5',6"-Tri-*O*-benzyl-3'-*O*-α-D-mannopyranosyl-2',3",4"-tri-*O*-*p*-methoxybenzyl-N^6-dimethoxytrityl adenosine (**106**)

105 (1.48g, 2.15mmol) And **40** (0.84g, 1.08mmol) in dioxane (12cm^3) and toluene (4cm^3) were stirred with 4Å molecular sieves (approx. 1.2g) for 2 h, and then dry zinc chloride (0.35g, 2.58mmol) and silver perchlorate (1.07g, 5.16mmol) were added. The flask was wrapped in foil and stirring was continued for 8 h. Solid $NaHCO_3$ (1.00g) and water (30cm^3) were added and the reaction mixture was diluted with ethyl acetate (40cm^3). After stirring for a further 30 min the mixture was filtered through a Celite pad, and the residue was well washed with ethyl acetate. Water (20cm^3) was added to the

filtrate, and the resulting aqueous layer was discarded. The organic layer was washed with brine ($50cm^3$), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7, then 1:1) to yield the title compound as a clear oil (0.89g, 61%).

R_f 0.53 (ethyl acetate/toluene 1:4); $[\alpha]_D^{18}$ –1.0 (*c* 1.0, $CHCl_3$); (Found: C, 72.10; H, 6.20; N, 5.06. Calc for $C_{82}H_{83}N_5O_{14}$: C, 72.28; H, 6.14; N, 5.14%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.55 (1H, 0.5 ABX, $^2J_{AB}$10.7, $^3J_{AX}$ 2.9, H-5'$_A$), 3.59–3.73 (4H, m, H-3" or H-4", H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.66 (3H, s, OCH_3), 3.77 (9H, s, 3 × OCH_3), 3.79 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.83–3.91 (3H, m, H-2", H-3" or H-4", H-5"), 4.28 (1H, dd, *J* 2.9, H-4'), 4.37–4.60 (13H, m, H-2', H-3', 11 × OCH*H*Ar), 4.77 (1H, AB, J_{AB} 10.3, OCH*H*Ar), 5.05 (1H, s, H-1"), 6.16 (1H, d, *J* 5.4, H-1'), 6.63–6.67 (2H, m, ArCH), 6.78–6.81 (4H, m, ArCH), 6.86–6.90 (2H, m, ArCH), 6.96–6.99 (2H, m, ArCH), 7.06–7.08 (2H, m, ArCH), 7.20–7.35 (28H, m, ArCH), 7.90, 8.07 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 55.07, 55.15, 55.20 (3q, 5 × OCH_3), 69.08 (t, C-6"), 69.52 (t, C-5'), 70.58 (s, DMTr Cq), 71.75, 72,03, 72.52, 73.29, 73.53, 74.70 (6d, 6 × O*C*H_2Ar), 72.37, 74.28, 74.59 (3d, C-3", C-4", C-5"), 73.21 (d, C-3"), 79.62 (d, C-2', C-2"), 82.56 (d, C-4'), 86.18 (d, C-1'), 97.81 (d, C-1"), 113.06, 113.65, 113.70 (3d, C-3 and C-5 of *p*-OCH_3 phenyl rings), 121.00 (s, C-5), 126.73, 127.40, 127.48, 127.55, 127.60, 127.73, 127.17, 128.24, 128.48, 128.76, 129.16, 129.49, 129.63, 130.05, 130.49 (15d, ArCH), 128.81, 130.51, 130.57(3s, C-1 of PMB rings), 137.42, 138.17 (2s, C-1 of DMTr *p*-OCH_3 phenyl rings, 3 × C-1 of Bn rings), 138.30 (d, C-8), 145.43 (s, C-1 of DMTr phenyl ring), 148.62 (s, C-4), 152.29 (d, C-2), 153.99 (s, C-6), 158.20 (s, 2 × C-4 of DMTr *p*-OCH_3 phenyl rings), 159.06, 159.11, 159.41 (3s, C-4 of PMB ring); *m/z* (FAB^+) 1362 (M^+, 7%), 436 (6), 303 (100), 121 (88).

7.5.3 2",5',6"-Tri-*O*-benzyl-3'-*O*-α-D-mannopyranosyl adenosine (**107**)

To **106** (528mg, 0.39mmol) in DCM ($27cm^3$), was added TFA ($3cm^3$). The resulting bright orange solution was stirred for 5 h before being poured into saturated aqueous $NaHCO_3$ solution ($200cm^3$). DCM ($100cm^3$) was added and the now colourless mixture was stirred vigorously for 30 min. The resulting aqueous layer was extracted with DCM

(2 × 100cm^3) and the combined organic layers dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate/ethanol 14:1, then 9:1) to yield the title compound as a white solid (223mg, 82%).

mp 175–178°C (ethanol); R_f 0.22 (ethyl acetate/ethanol 14:1); (Found: M^+, 700.296. Calc for $C_{37}H_{42}N_5O_9$ $(M+H)^+$: 700.298); ^{1}H NMR (400 MHz; D_6 DMF) δ_H 3.68–3.86 (6H, m, H-4", H-5", H-5'$_A$, H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.93–3.96 (1H, m, H-3"), 3.99–4.00 (1H, m, H-2"), 4.39 (1H, ddd, *J* 3.2, H-4'), 4.34–4.62 (5H, m, 2 × OC*H*$_2$Ar, H-3'), 4.71, 4.80 (2H, AB, J_{AB} 12.2, OC*H*$_2$Ar), 4.97–5.03 (2H, m, H-2', 3"-OH), 5.13 (1H, d, *J* 4.7, 4"-OH), 5.37 (1H, s, H-1"), 5.95 (1H, d, *J* 6.7, 2'-OH), 6.11 (1H, d, *J* 6.4, H-1'), 7.24–7.44 (15H, m, ArCH), 8.22, 8.34 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; d_6 DMF) δ_C 68.83 (d, C-4" or C-5"), 71.12, 71.24 (2t, C-5', C-6"), 72.49 (d, C-3"), 73.47, 73.63, 73.73 (3t, 3 × O*C*H$_2$Ar), 74.53 (d, C-4" or C-5"), 74.72 (d, C-2'), 76.59 (d, C-3'), 79.62 (d, C-2"), 83.35 (d, C-4'), 88.10 (d, C-1'), 99.20 (d, C-1"), 120.18 (s, C-5), 127.86, 127.96, 128.00, 128.11, 128.24, 128.77, 128.91, 129.03 (8d, ArCH), 139.21, 139.21, 139.86 (3s, 3 × C-1 of Bn ring), 140.05 (d, C-8), 150.85 (s, C-4), 153.62 (d, C-2), 157.14 (s, C-6); *m/z* (FAB$^+$) 700 [$(M+H)^+$, 70%], 91 (100).

7.5.4 2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-D-mannopyranosyl adenosine (**108**)

A solution of **107** (100mg, 0.14mmol) in DCM (3cm^3) with bis(benzyloxy)(diisopropylamino)phosphine (0.16cm^3, 0.49mmol) and imidazolium triflate (103mg, 0.47mmol), was stirred for 1 h, after which time TLC (ethyl acetate/hexane 7:3) indicated conversion to the trisphosphite (R_f 0.67). Water (1 drop) was added and the solution cooled to -78°C, after which MCPBA (144mg, 0.50mmol) was added. After 10 min 10% aqueous Na_2SO_3 solution (15cm^3) and ethyl acetate (20cm^3) were added and the mixture was allowed to warm to room temperature. The resulting organic layer was washed with 15cm^3 each of saturated aqueous $NaHCO_3$ solution and brine, and then dried ($MgSO_4$), filtered and concentrated to a clear oil which

was subjected to flash chromatography (eluent chloroform/acetone 9:1, then 4:1, then 3:2) to give the title compound as a clear oil (119mg, 56%).

R_f 0.29 (ethyl acetate/hexane 7:3); $[\alpha]_D^{20}$ –1.9 (*c* 1.1, $CHCl_3$); (Found: M^+, 1480.483. Calc for $C_{79}H_{81}N_5O_{18}P_3$ $(M-H)^-$: 1480.478); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.53, 3.66 (2H, ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 3.2, $^3J_{BX}$ 2.5, H-5'$_A$, H-5'$_B$), 3.70 (1H, 0.5 ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 5.9, H-6"$_A$), 3.75–3.78 (1H, m, H-6"$_B$), 3.82–3.86 (1H, m, H-5"), 4.26–4.29 (1H, m, H-4'), 4.37–4.44 (5H, m, H-2", 2 × OCH_2Ar), 4.54, 4.64 (2H, AB, J_{AB} 11.7, OCH_2Ar), 4.68 (1H, dd, *J* 4.7, H-3'), 4.79–5.03 (14H, m, 6 × OCH_2Ar, H-3", H-4"), 5.30 (1H, d, *J* 1.5, H-1"), 5.44 (1H, ddd, *J* 3.5, J_{H-P} 8.5, H-2'), 5.86 (2H, br.s, NH_2), 6.22 (1H, d, *J* 5.0, H-1'), 7.11–7.32 (45H, m, ArCH), 8.00, 8.23 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 69.24–70.34 (8t, C-5', C-6", 6 × POCH_2Ar with C-P coupling), 72.05 (d, C-5" with C-P coupling), 72.87 (d, C-4" with C-P coupling), 72.96, 73.60, 73.88 (3t, 3 × OCH_2Ar), 74.49 (d, C-4"), 76.28 (d, C-3" with C-P coupling), 76.77 (d, C-2"), 77.67 (d, C-2'), 82.27 (d, C-4'), 86.84 (d, C-1' with C-P coupling), 97.75 (d, C-1"), 119.94 (s, C-5), 127.50, 127.59, 127.72, 127.87, 127.96, 127.97, 128.05, 128.08, 128.13, 128.20, 128.32, 128.39, 128.49, 128.61, 128.64, 128.69, 128.73 (17d, ArCH), 135.31–135.95 (6s, 6 × C-1 of benzylphospho ring with C-P coupling), 137.42, 138.14, 138.39 (3s, 3 × C-1 of Bn rings), 139.15 (d, C-8), 149.92 (s, C-4), 153.08 (d, C-2), 155.38 (s, C-6); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^1H decoupled) δ_P -1.30, -0.80, -0.35 (3s); *m/z* (FAB$^+$) 1480 [$(M+H)^+$, 3%], 91 (100).

7.5.5 3-O-α-D-Mannopyranosyl adenosine 2',3",4"-trisphosphate (**97**)

A mixture of **108** (61mg, 0.04mmol) and wet 20% palladium hydroxide on carbon (180mg), in methanol (7.2cm^3), cyclohexane (3.6cm^3) and water (0.7cm^3) was heated at reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed copiously with methanol and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion exchange resin column and eluting with a gradient of 0–100% 150mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20°C) gave the desired product as the free acid (19mg, 71%), which was dissolved

in water and eluted through a short column of Na^+ dianion WK-40 ion exchange resin to give, after concentration, the sodium salt.

(Found: M^-, 668.039. Calc for $C_{16}H_{25}N_5O_{18}P_3$ $(M-H)^-$: 668.040); ^1H NMR (400 MHz; D_2O) δ_H 3.58–3.72 (5H, m, H-5'$_A$, H-5'$_B$, H-5", H-6"$_A$, H-6"$_B$), 4.15–4.24 (3H, m, H-2', H-4', H-4"), 4.39–4.48 (2H, m, H-3', H-3"), 4.98 (1H, s, H-1"), 5.08–5.14 (1H, m, H-2'), 6.11 (1H, d, *J* 6.4, H-1'), 8.24, 8.33 (2H, 2s, H-2, H-8); ^{31}P NMR (161.7 MHz; D_2O; ^1H decoupled) δ_P 0.12, 0.49, 0.86 (3s); UV (H_2O) λ_{max} 259nm, ε 15 400, pH 7.5; *m/z* (FAB^-) 668 [$(M-H)^-$, 100%].

7.6 Synthesis of xylophostin

7.6.1 Allyl α-D-xylopyranoside (**109**)

Allyl alcohol (1000cm^3) and acetyl chloride (6cm^3) were stirred together for 30 min, after which time D-xylose (100.00g, 0.67mol) was added. The mixture was refluxed for 16 h then cooled. Solid $NaHCO_3$ (7.00g) was slowly added, and stirring continued for 30 min. The mixture was then filtered and the filtrate was concentrated to an off-white solid. The solid was dissolved in a minimum of ethanol (700cm^3) and the solution was kept at -20°C for 24 h. Diisopropyl ether was added immediately before the collection of the title compound over three crops as white fluffy crystals (52.25g, 41%). R_f 0.47 (ethyl acetate/methanol 9:1); mp 100–102°C (ethanol) [lit.[88] 101–103°C]; ^{1}H NMR (400 MHz; D_2O) δ_H 3.51–3.70 (5H, m, H-2, H-3, H-4, H-5$_A$, H-5$_B$), 4.01–4.24 (2H, m, CH_2CH=CH$_2$), 4.92 (1H, d, J 3.5, H-1), 5.25 (1H, m, ^3J 10.3, $CH_2CH=CH_{cis}CH_{trans}$), 5.35 (1H, d, ^3J 17.2, $CH_2CH=CH_{cis}CH_{trans}$), 5.90–6.04 (1H, m, $CH_2CH=CH_2$).

7.6.2 Allyl 2-*O*-pivaloyl-α-D-xylopyranoside (**116**)

A solution of **109** (4.00g, 21.05mmol) in pyridine (30cm^3) was cooled to -20°C and pivaloyl chloride (2.56cm^3, 20.79mmol) was added dropwise. TLC (ethyl acetate) indicated conversion of starting material to one major product (R_f 0.74). The reaction mixture was partitioned between 0.25M aqueous HCl (100cm^3) and ethyl acetate (200cm^3), and the resulting organic layer was washed with saturated aqueous $NaHCO_3$ solution (100cm^3) and brine (100cm^3), dried ($MgSO_4$), filtered and concentrated to give the title compound of sufficient purity to be used in the next step. A small amount of the residue was purified by flash chromatography (eluent ether/hexane 3:2) to yield an analytical sample as a clear oil which crystallised on standing, while the remaining crude product was carried forward to the next reaction.

mp 73–75°C (hexane); $[\alpha]_D^{17}$ +164 (*c* 1.0, $CHCl_3$); (Found: C, 57; H, 8.16. Calc for $C_{13}H_{22}O_6$: C, 56.92; H, 8.08%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.23 (9H, s, C(O)C(CH_3)$_3$), 3.27 (1H, br. s, D_2O exch., 3-OH), 3.51 (1H, br. s, D_2O exch., 4-OH), 3.56 (1H, dd, $^2J = {}^3J$ 12.0, H-5_{ax}), 3.67–3.74 (2H, m, H-4, H-5_{eq}), 3.91–3.96 (2H, m, H-3, C*H*HCH=CH_2), 4.15–4.20 (1H, m, CH*H*CH=CH_2), 4.61 (1H, dd, *J* 3.8, 10.0, H-2), 4.97 (1H, d, *J* 3.5, H-1), 5.18 (1H, m, ^3J 10.2, CH_2CH=C*H*$_{cis}$$CH_{trans}$), 5.29 (1H, m, ^3J 17.1, CH_2CH=CH_{cis}C*H*$_{trans}$), 5.78–5.89 (1H, m, CH_2C*H*=CH_2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 27.46 (q, C(O)C(CH_3)$_3$), 39.27 (s, C(O)*C*(CH_3)$_3$), 61.65 (t, C-5), 68.69 (t, *C*H_2CH=CH_2), 70.74 (d, C-4), 72.55 (d, C-3), 73.40 (d, C-2), 95.47 (d, C-1), 117.71 (t, CH_2CH=*C*H_2), 113.66 (d, CH_2*C*H=CH_2), 178.63 (s, *C*(O)C(CH_3)$_3$); *m/z* (FAB$^+$) 275 [(M+H)$^+$, 22%], 32 (297), 217 (100), 199 (43), 85 (22).

7.6.3 Allyl 3,4-*O*-isopropylidene-2-*O*-pivaloyl-α-D-xylopyranoside (**117**)

To crude **116** (5.46g, 12.41mmol) in THF (30cm^3) was added 2-methoxypropene (2.74cm^3, 28.64mmol) and *p*TSA (50mg). After stirring for 1 h TLC (ether/hexane 1:6) indicated conversion to one product (R_f 0.29). The reaction mixture was diluted with ether (150cm^3) and the organic solution washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), filtered and concentrated. Flash chromatography (eluent ether/hexane 1:7, then 1:6) to yielded the title compound as a clear runny oil (3.38g, 51%, over two steps based on allyl α-D-xylopyranoside).

$[\alpha]_D^{20}$ +143.2 (*c* 0.4, $CHCl_3$); (Found: C, 61.00; H, 8.29. Calc for $C_{16}H_{26}O_6$: C, 61.13; H, 8.34%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.23 (9H, s, C(O)C(*C*H_3)$_3$), 1.46 (6H, s, 2 × isopropylidene CH_3), 3.48–3.54 (1H, m, H-4), 3.76 (1H, dd, $^2J = {}^3J$ 10.2, H-5_{ax}), 3.90–3.96 (3H, m, H-3, H-5_{eq}, C*H*HCH=CH_2), 4.16–4.21 (1H, m, CH*H*CH=CH_2), 4.82 (1H, dd, *J* 3.8, H-2), 5.16 (1H, d, *J* 3.8, H-2), 5.19 (1H, m, ^3J 10.2, CH_2CH=C*H*$_{cis}$$CH_{trans}$), 5.29 (1H, m, ^3J 17.3, CH_2CH=CH_{cis}C*H*$_{trans}$), 5.80–5.90 (1H, m, CH_2C*H*=CH_2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.54, 26.81, 27.10 (3q, 2 × isopropylidene CH_3, C(O)C(*C*H_3)$_3$), 38.77 (s, C(O)*C*(CH_3)$_3$), 61.27 (t, C-5), 68.96 (t, *C*H_2CH=CH_2), 72.88 (d, C-4), 74.12 (d, C-3), 75.75 (d, C-2), 95.10 (d, C-1), 110.86 (s,

isopropylidene $C(CH_3)_2$), 117.83 (t, $CH_2CH=CH_2$), 133.50 (d, $CH_2CH=CH_2$), 177.96 (s, $C(O)C(CH_3)_3$); *m/z* (FAB^+) 315 [$(M+H)^+$, 49%], 57 (100).

7.6.4 Allyl 3,4-*O*-isopropylidene-α-D-xylopyranoside (**118**)

A solution of **117** (3.07g, 9.78mmol) in methanol (40cm^3) containing sodium hydroxide pellets (1.00g) was refluxed for 90 min. After cooling the sodium hydroxide was neutralised by careful addition of solid CO_2. The solvent was then evaporated under reduced pressure and DCM was added to the residue (250cm^3), remaining solid $NaHCO_3$ was removed by filtration and the filtrate concentrated to a clear oil which was subjected to flash chromatography (eluent ether/hexane 1:1) to yield the title compound as a clear runny oil (2.03g, 90%).

$[\alpha]_D^{20}$ +133.7 (*c* 0.4, $CHCl_3$); (Found: C, 57.10; H, 7.94. Calc for $C_{11}H_{18}O_5$: C, 57.38; H, 7.94%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.45, 1.46 (6H, 2s, 2 × isopropylidene CH_3), 2.43 (1H, d, *J* 10.8, 2-OH), 3.38–3.44 (1H, m, H-4), 3.66–3.74 (2H, m, H-5_{ax}, H-5_{equ}), 3.83 (1H, ddd, *J* 4.1, 10.4, H-2), 3.94 (1H, dd, *J* 4.4, 9.7, H-3), 4.02–4.07 (1H, m, C*H*HCH=CH_2), 4.23–4.28 (1H, m, CH*H*CH=CH_2), 4.95 (1H, d, *J* 3.8, H-1), 5.24 (1H, m, ^3J 10.2, $CH_2CH=CH_{cis}CH_{trans}$), 5.32 (1H, m, ^3J 17.3, $CH_2CH=CH_{cis}CH_{trans}$), 5.87–5.97 (1H, m, $CH_2CH=CH_2$); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.40, 26.73 (2q, 2 × isopropylidene CH_3), 61.57 (t, C-5), 68.84 (t, $CH_2CH=CH_2$), 71.87, 73.62, 79.33 (3d, C-4, C-3, C-2), 97.50 (d, C-1), 110.70 (s, isopropylidene $C(CH_3)_2$), 118.07 (t, $CH_2CH=CH_2$), 133.35 (d, $CH_2CH=CH_2$); *m/z* (FAB^+) 231 [$(M+H)^+$, 100%], 59 (75).

7.6.5 Allyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-D-xylopyranoside (**119**)

Sodium hydride (0.59g of a 60%^{w}/w dispersion in mineral oil, 14.71mmol) was added to **118** (1.78g, 7.74mmol) in DMF (20cm^3). Thirty min later benzyl bromide (1.10cm^3, 8.83mmol) was added and stirring was continued overnight. The reaction mixture was partitioned between ether (100cm^3) and water (80cm^3) and the aqueous layer was extracted with ether (100cm^3). The combined ethereal layers were dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash

chromatography (eluent ether/hexane 1:9) to yield the title compound as a clear oil (2.41g, 97%).

R_f 0.19 (ether/hexane 1:9); $[\alpha]_D^{20}$ +30.6 (*c* 0.5, $CHCl_3$); (Found: C, 67.40; H, 7.53. Calc for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.46, 1.47 (6H, 2s, 2 × isopropylidene CH_3), 3.35–3.41 (1H, m, H-4), 3.65 (1H, dd, *J* 3.5, 10.2, H-2), 3.73 (1H, dd, $^2J = {}^3J$ 10.2, H-5_{ax}), 3.86 (1H, dd, ^2J 9.7, ^3J 4.7, H-5_{eq}), 3.91–4.00 (2H, m, H-3, C*H*HCH=CH_2), 4.15–4.20 (1H, m, CH*H*CH=CH_2), 4.64 (1H, AB, J_{AB} 12.3, OC*H*HAr), 4.83–4.86 (2H, m, H-1, OCH*H*Ar), 5.21 (1H, m, ^3J 10.2, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.34 (1H, m, ^3J 17.3, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.86–5.96 (1H, m, $CH_2CH{=}CH_2$), 7.24–7.39 (5H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.43, 26.82 (2q, 2 × isopropylidene CH_3), 61.09 (t, C-5), 68.44 (t, $CH_2CH{=}CH_2$), 71.82 (t, OCH_2Ar), 74.13, 77.91, 77.97 (3d, C-4, C-3, C-2), 96.24 (d, C-1), 110.54 (s, isopropylidene $C(CH_3)_2$), 117.80 (t, $CH_2CH{=}CH_2$), 127.62, 127.86, 128.26 (3d, ArCH), 133.69 (d, $CH_2CH{=}CH_2$), 138.05 (s, C-1 of Bn ring); *m/z* (FAB^+) 321 [$(M+H)^+$, 14%], 91 (100).

7.6.6 Allyl 2-*O*-benzyl-α-D-xylopyranoside (**113**)

A solution of allyl **119** in methanol (30cm^3) containing 1M aqueous HCl (3cm^3) was stirred for 20 min after which time TLC (ether/hexane 4:1) indicated conversion of starting material to one product (R_f 0.17). Solid $NaHCO_3$ was carefully added until effervescing ceased and the reaction mixture was concentrated under reduced pressure. The residue was partitioned between DCM (100cm^3) and water (75cm^3), and the aqueous layer extracted with DCM (3 × 50cm^3). The combined organic layers were dried ($MgSO_4$), filtered and concentrated and the remaining crude product subjected to flash chromatography (eluent ether/hexane 9:1) to yield the title compound as a clear oil that slowly crystallised on standing (1.70g, 97%).

mp 42–44°C (diisopropyl ether) [lit.[88] reported no crystals]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.31 (1H, dd, *J* 3.5, 9.5, H-5), 3.45–3.60 (2H, m, H-5_{ax}, H-5_{equ}), 3.85–3.94 (2H, m, H-3, C*H*HCH=CH_2), 4.11–4.18 (1H, m, CH*H*CH=CH_2), 4.63, 4.64 (2H, AB, J_{AB} 12.1, OCH_2Ar), 4.76 (1H, d, *J* 3.5, H-1), 5.20 (1H, m, ^3J 10.2, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.31

(1H, m, ^3J 17.3, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.83–5.97 (1H, m, $CH_2CH{=}CH_2$), 7.28–7.38 (5H, m, ArCH).

7.6.7 Allyl 2-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-xylopyranoside (**114**)

Sodium hydride (2.86g of a 60%^{w}/w dispersion in mineral oil, 71.43mmol) was added to **113** (5.00g, 17.86mmol) in DMF (50cm^3). Twenty min later *p*-methoxybenzyl chloride (9.69cm^3, 71.43mmol) was added and stirring was continued overnight. The reaction mixture was quenched with water (2cm^3) and then partitioned between ether (200cm^3) and water (150cm^3), the aqueous layer was extracted with ether (2 × 150cm^3) and the combined ethereal layers washed with water (100cm^3) before being dried ($MgSO_4$), filtered and concentrated. The remaining crude product was purified by flash chromatography (eluent ether/hexane 3:7) to yield the title compound as a clear oil (7.84g, 84%).

R_f 0.59 (ether/hexane 3:2); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.43 (1H, *J* 3.7, 9.5, H-2), 3.49–3.57 (2H, m, $H\text{-}5_{ax}$, $H\text{-}5_{equ}$), 3.78, 3.79 (6H, 2s, 2 × OCH_3), 3.87–3.92 (1H, m, H-3), 3.95–4.00 (1H, m, $CHHCH{=}CH_2$), 4.13–4.17 (1H, m, $CHHCH{=}CH_2$), 4.54, 4.68 (2H, AB, J_{AB} 11.2, OCH_2Ar), 4.64, 4.77 (2H, AB, J_{AB} 12.2, OCH_2Ar), 4.70 (1H, d, *J* 3.4, H-1), 4.79, 4.84 (2H, AB, J_{AB} 10.3, OCH_2Ar), 5.21 (1H, m, ^3J 10.2, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.31 (1H, m, ^3J 17.3, $CH_2CH{=}CH_{cis}CH_{trans}$), 5.88–5.97 (1H, m, $CH_2CH{=}CH_2$), 6.84–6.87 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.23–7.36 (9H, m, ArCH).

7.6.8 2-*O*-Benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-xylopyranose (**115**)

A solution of **114** (6.87g, 13.21mmol) in methanol (60cm^3) was cooled to 0°C and palladium chloride (0.47g, 2.64mmol) was added. The flask was fitted with a drying tube, the cooling bath removed and the reaction mixture was stirred vigorously for 4 h after which time TLC (ether/hexane 3:2) indicated conversion of starting material to

product (R_f 0.24). The reaction mixture was quenched with triethylamine and filtered through a Celite pad. The filtrate was concentrated and the dark brown residue subjected to flash chromatography (eluent ether/hexane 3:2) to yield the title compound as a white solid (5.72g, 90%).

mp 77–80°C [lit.[88] 88–90°C]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.20–3.30 (0.6H, m, H-2$_\beta$, H-4$_\beta$), 3.45 (0.7H, dd, *J* 3.7, 8.9, H-2$_\alpha$), 3.47–3.91 (3.7H, m, H-3, H-4$_\alpha$, H-5$_{ax}$, H-5$_{equ}$), 3.79, 3.80 (6H, 2s, OCH_3), 4.53–4.89 (6.3H, m, 3 × OCH_2Ar, H-1$_\beta$), 5.08 (0.7H, d, *J* 3.5, H-1$_\alpha$), 6.83–6.88 (4H, m, 2 × H-3 and H-5 of PMB rings), 7.22–7.37 (9H, m, ArCH). α and β subscripts denote signals arising from α and β-anomers respectively.

7.6.9 2-*O*-Benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-xylopyranosyl dimethyl phosphite (**120**)

To a mixture of **115** (1.23g, 2.57mmol) and tetrazole (0.27g, 3.85mmol) in DCM (12cm^3) was added bis(methoxy)(diethylamino)phosphine (0.55cm^3, 3.34mmol). After stirring for 20 min TLC (ethyl acetate/toluene 1:4) indicated complete conversion to product (R_f 0.63). The reaction mixture was partitioned between diethyl ether (100cm^3) and water (75cm^3). The resulting ethereal layer was washed with brine (75cm^3), dried ($MgSO_4$), filtered and concentrated to give a clear runny oil that was used without further purification.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.23 (0.6H, ddd, *J* 9.8, H-4$_\beta$), 3.40–3.95 (11.4H, m, H-2, H-3, H-4$_\alpha$, H-5$_A$, H-5$_B$, P(OCH_3)$_2$ with C-P coupling), 3.77 (3.6H, s, $OCH_{3\alpha}$), 3.77 (2.4H, s, $OCH_{3\beta}$), 4.54–4.90 (6.6H, H-1$_\beta$, 3 × OCH_2Ar), 5.42 (0.4H, dd, *J* 3.4, $J_{H\text{-}P}$ 8.6, H-1$_\alpha$), 6.81–6.89 (4H, m, ArCH), 7.22–7.37 (9H, m, ArCH); α and β subscripts denote signals arising from α and β-anomers respectively; ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 140.88 OP$_\beta$(OMe)$_2$, 142.05 OP$_\alpha$(OMe)$_2$.

7.6.10 2",5'-Di-*O*-benzyl-2',3",4"-tri-*O*-*p*-methoxybenzyl-N^6-dimethoxytrityl-3'-*O*-α-D-xylopyranosyl adenosine (**121a**)

120 (1.47g, 2.57mmol) And **40** (1.00g, 1.28mmol) in dioxane (15cm^3) and toluene (5cm^3) were stirred with 4Å molecular sieves (approx. 1.50g) for 2 h, and then dry zinc chloride (0.42g, 3.08mmol) and silver perchlorate (1.26g, 6.16mmol) were added. The flask was wrapped in foil and stirring was continued for 9 h. Solid $NaHCO_3$ (1.00g) and water (30cm^3) were added and the reaction mixture was diluted with ethyl acetate (40cm^3). After stirring for a further 30 min the mixture was filtered through a Celite pad, and the residue well washed with ethyl acetate. Water (30cm^3) was added to the filtrate and the resulting aqueous layer was discarded. The organic layer was washed with brine (75cm^3), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7, then 2:3, then 1:1) to yield the title compound and its β-xylopyranosyl anomer in an inseparable 1:1 mixture (0.76g, 46%).

R_f 0.38 (ethyl acetate/toluene 1:4); Selected ^{1}H NMR data for H-1' of the α and β coupled products: ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 6.13 (0.5H, d, *J* 1.5), 6.22 (0.5H, d, *J* 4.4); *m/z* (FAB$^+$) 1246 [(M+H)$^+$, 14%], 303 (100), 121 (72).

7.6.11 2",5'-Di-*O*-benzyl-3'-*O*-α-D-xylopyranosyl adenosine (**122a**)

To **121ab** (745mg, 0.60mmol) in DCM (27cm^3), was added TFA (3cm^3). The resulting bright orange solution was stirred for 1 h before being poured into saturated aqueous $NaHCO_3$ solution (150cm^3). DCM (75cm^3) was added and the now colourless mixture was stirred vigorously for 30 min. The resulting aqueous layer was extracted with DCM (2 × 75cm^3) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate/methanol 14:1) to yield 2",5'-di-*O*-benzyl-3'-*O*-α-D-xylopyranosyl adenosine (138mg, 40%), with further elution furnishing 2",5'-di-*O*-benzyl-3'-*O*-β-D-xylopyranosyl adenosine (143mg, 41%).

Analyses for 2",5'-di-*O*-benzyl-3'-*O*-α-D-xylopyranosyl adenosine (**122a**).

R_f 0.51 (chloroform/methanol 9:1); $[\alpha]_D^{18}$ +22.1 (*c* 2.0, acetone); (Found: M^+, 580.240. Calc for $C_{29}H_{34}N_5O_8$ $(M+H)^+$: 580.240); ^1H NMR (400 MHz; $(CD_3)_2CO$) δ_H 3.12 (1H, br. s, OH), 3.44 (1H, dd, *J* 3.7, 9.5, H-2"), 3.60–3.65 (3H, m, H-4", H-5"$_{ax}$, H-5"$_{equ}$), 3.74, 3.83 (2H, ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 3.8, $^3J_{BX}$ 3.4, H-5'$_A$, H-5'$_B$), 3.95–3.99 (1H, m, H-3"), 4.36 (1H, ddd, *J* 4.1, H-4'), 4.57–4.63 (4H, m, OC*H*$_2$Ar), 4.71 (1H, br s, OH), 4.84–4.88 (1H, m, H-2' obscured by HDO peak), 5.03 (1H, br s, OH), 5.17 (1H, d, *J* 3.8, H-1"), 6.12 (1H, d, *J* 4.7, H-1'), 6.98 (2H, br s, NH_2), 7.19–7.43 (10H, m, ArCH), 8.22, 8.26 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $(CD_3)_2CO$) δ_C 62.82 (t, C-5"), 69.91 (t, C-5'), 70.59 (d, C-4"), 73.34, 73.66 (2t, 2 × O*C*H$_2$Ar), 73.96 (d, C-3"), 74.44 (d, C-2'), 77.47 (d, C-3'), 79.70 (d, C-2"), 82.29 (d, C-4'), 88.66 (d, C-1'), 98.58 (d, C-1"), 119.65 (s, C-5), 127.72, 127.81, 128.33, 128.40, 128.51 (5d, ArCH), 138.40, 138.43 (2s, 2 × C-1 of Bn rings), 139.39 (d, C-8), 149.89 (s, C-4), 153.04 (d, C-2), 156.28 (s, C-6); *m/z* (FAB^+) 580 $[(M+H)^+$, 100%], 91 (79).

Analyses for 2",5'-di-*O*-benzyl-3'-*O*-β-D-xylopyranosyl adenosine (**122b**).

R_f 0.43 (chloroform/methanol 9:1); (Found: M^+, 580.241. Calc for $C_{29}H_{34}N_5O_8$ $(M+H)^+$: 580.240); ^1H NMR (400 MHz; CD_3OD) δ_H 3.18 (1H, dd, $^2J = {}^3J$ 10.8, H-5"$_{ax}$), 3.29 (1H, dd, *J* 7.6, 9.1, H-2"), 3.46 (1H, dd, *J* 8.9, H-3"), 3.53–3.58 (1H, m, H-4"), 3.61, 3.77 (2H, ABX, $^2J_{AB}$ 11.0, $^3J_{AX}$ 3.1, $^3J_{BX}$ 2.8, H-5'$_A$, H-5'$_B$), 3.85 (1H, dd, ^2J 11.4, ^3J 5.3, H-5"$_{equ}$), 4.34 (1H, ddd, *J* 2.9, 5.6, H-4'), 4.43, 4.48 (2H, AB, J_{AB} 12.0, OC*H*$_2$Ar), 4.46 (1H, d, *J* 7.9, H-1"), 4.53 (1H, dd, *J* 5.1, H-3'), 4.66 (1H, dd, *J* 4.2, H-2'), 4.78, 4.86 (2H, AB, J_{AB} 11.1, OC*H*$_2$Ar), 6.11 (1H, d, *J* 3.8, H-1'), 7.19–7.39 (10H, m, ArCH), 8.18, 8.30 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; CD_3OD) δ_C 67.17 (t, C-5"), 70.30 (t, C-5'), 71.22 (d, C-4"), 74.63, 75.97 (2t, O*C*H$_2$Ar), 76.11 (d, C-2'), 77.62 (d, C-3"), 79.79 (d, C-3'), 82.81 (d, C-2"), 83.12 (d, C-4'), 90.27 (d, C-1'), 105.19 (d, C-1"), 120.31 (s, C-5), 128.62, 128.97, 128.01, 129.33, 129.61 (5d, ArCH), 139.14, 140.13 (2s, 2 × C-1 of Bn rings), 140.63 (d, C-8), 150.36 (s, C-4), 153.91 (d, C-2), 157.11 (s, C-6); *m/z* (FAB^+) 580 $[(M+H)^+$, 100%], 91 (67).

7.6.12 2",5'-Di-O-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-O-α-D-xylopyranosyl adenosine (**123**)

A solution of **122a** (101mg, 0.17mmol) in DCM (3.5cm^3) with bis(benzyloxy)(diisopropylamino)phosphine (0.19cm^3, 0.58mmol) and imidazolium triflate (125mg, 0.58mmol), was stirred for 1 h, after which time TLC (ethyl acetate/hexane 7:3) indicated some starting material remaining; therefore a further 1.0 equivalent each of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate was added. TLC after a further 30 min indicated conversion to the trisphosphite (R_f 0.63). Water (1 drop) was added and the solution cooled to -78°C, after which MCPBA (170mg, 0.59mmol) was added. After 10 min 10% aqueous Na_2SO_3 solution (15cm^3) and ethyl acetate (20cm^3) were added and the mixture was allowed to warm to room temperature. The resulting organic layer was washed with 15cm^3 each of saturated aqueous $NaHCO_3$ solution and brine, and then dried ($MgSO_4$), filtered and concentrated to a clear oil which was subjected to flash chromatography (eluent chloroform/acetone 9:1, then 7:1, then 6:1, then 4:1, then 3:2) to give the title compound as a clear oil (161mg, 68%).

R_f 0.11 (ethyl acetate/hexane 7:3); $[\alpha]_D^{20}$ +6.5 (*c* 1.7, $CHCl_3$); (Found: M^+, 1360.425. Calc for $C_{71}H_{73}N_5O_{17}P_3$ $(M+H)^+$: 1360.421); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.49 (1H, dd, *J* 3.5, 9.4 H-2"), 3.52 (1H, dd, $^2J = {}^3J$ 11.0, H-5"$_{ax}$), 3.62, 3.76 (2H, ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 3.2, $^3J_{BX}$ 3.4, H-5'$_A$, H-5'$_B$), 3.95 (1H, dd, ^2J 11.1, ^3J 5.9, H-5"$_{equ}$), 4.33–5.02 (14H, m, H-3', H-3", H-4', H-4", 6 × POC*H*$_2$Ar, 2 × OC*H*$_2$Ar), 5.29 (1H, d, *J* 3.2, H-1"), 5.61–5.66 (1H, m, H-2"), 5.98 (2H, br. s, NH_2), 6.34 (1H, d, *J* 5.9, H-1'), 6.98–7.39 (40H, m, ArCH), 7.93, 8.25 (2H, 2s, H-2, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 60.25 (t, C-5"), 69.40–70.23 (7t, C-5', 6 × POCH_2Ar with C-P coupling), 72.12 (t, OCH_2Ar), 73.59 (d, C-4" with C-P coupling), 73.97 (t and d, OCH_2Ar and C-3'), 77.07 (d, C-2"), 77.71 (2d, C-2', C-3" with C-P coupling), 82.55 (d, C-4'), 86.01 (d, C-1'), 95.81 (d, C-1"), 120.05 (s, C-5), 127.78, 127.91, 127.98, 128.15, 128.19, 128.22, 128.26, 128.34, 128.48, 128.53, 128.68, 128.72, 128.79 (13d, ArCH), 135.14–136.23 (6s, 6 × C-1 of benzylphospho rings), 137.14, 137.64 (2s, 2 × C-1 of Bn rings), 139.54 (d, C-8), 150.14 (s, C-4), 153.14 (d, C-2), 155.54 (s, C-6); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P -0.63 (s, 2P), -0.20 (2s); *m/z* (FAB$^+$) 1360 [$(M+H)^+$, 5%], 91 (100).

7.6.13 3'-O-α-D-xylopyranosyl adenosine 2',3",4"-trisphosphate (**98**)

A mixture of **123** (84mg, 0.06mmol) and wet 20% palladium hydroxide on carbon (252mg), in methanol (11cm^3), cyclohexane (5cm^3) and water (1cm^3) was heated at reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst washed copiously with methanol and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion exchange resin column and eluting with a gradient of 0–100% 150mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20°C) gave the desired product as the free acid (33mg, 85%), which was dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion exchange resin to give, after concentration, the sodium salt.

(Found: M^-, 638.030. Calc for $C_{15}H_{23}N_5O_{17}P_3$ $(M-H)^-$: 638.030); ^{1}H NMR (400 MHz; D_2O) δ_H 3.50 (1H, dd, $^2J = {}^3J$ 10.8, H-5"$_{ax}$), 3.61 (1H, dd, J 3.4, 9.2, H-2"), 3.65–3.71 (2H, m, H-5'$_A$, H-5'$_B$), 3.76 (1H, dd, J 5.6, 11.4, H-5"$_{equ}$), 4.02–4.10 (1H, m, H-4"), 4.25–4.32 (2H, m, H-3", H-4"), 4.34–4.56 (1H, m, H-3'), 5.07 (1H, d, J 3.5, H-1"), 5.08–5.14 (1H, m, H-2'), 6.16 (1H, d, J 6.2, H-1'), 8.23, 8.33 (2H, 2s, H-2, H-8); ^{31}P NMR (161.7 MHz; D_2O; ^{1}H decoupled) δ_P 0.23, 0.67, 0.78 (3s); UV (H_2O) λ_{max} 259nm, ε 15 400, pH 7.5; *m/z* (FAB$^-$) 638 [$(M-1)^-$, 100%].

7.7 Synthesis of a versatile disaccharide intermediate

7.7.1 3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-D-xylofuranose (**126**)

A solution of 1,2-*O*-isopropylidene-α-D-xylofuranose (2.00g, 10.52mmol), benzaldehyde dimethyl acetal (1.74cm^3, 11.04mmol) and PTSA (54mg, 0.53mmol) in DMF (40cm^3) was heated at 70°C in a flask fitted with an air condenser attached to a water pump. After heating for 2h under reduced pressure the reaction mixture was cooled and saturated aqueous $NaHCO_3$ solution (20cm^3) and water (40cm^3) were added. The resulting mixture was extracted with ether (3 × 40cm^3), and the combined ethereal extracts were dried ($MgSO_4$), filtered and concentrated and the title compound was isolated as fine white needle-like crystals by crystallisation from ethanol (2.18g). Subjection of the mother liquor to flash chromatography, (eluent chloroform/acetone 9:1, 1:2 with pentane) gave the further product (0.42g), total yield (2.60g, 89%).
mp 125°C (ethanol); $[\alpha]_D^{18}$ +3.8 (*c* 1.1, $CHCl_3$); (Found: C, 64.6; H, 6.57. Calc for $C_{15}H_{19}O_5$: C, 64.74; H, 6.52%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.33, 1.52 (6H, 2s, 2 × isopropylidene CH_3), 4.12–4.16 (2H, m, H-5$_A$, H-5$_B$), 4.42–4.47 (2H, m, H-4, H-3), 4.64 (1H, d, *J* 3.4, H-2), 5.45 (1H, s, C*H*Ar), 6.07 (1H, d, *J* 3.9, H-1), 7.33–7.48 (5H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.17, 26.74 (2q, 2 × isopropylidene CH_3), 66.79 (t, C-5), 72.20 (d, C-3 or C-4), 79.04 (d, C-2), 83.87 (d, C-3 or C-4), 99.34 (d, benzylidene *C*H), 105.69 (d, C-1), 111.81 (s, isopropylidene *C*$(CH_3)_2$), 126.10. 128.25, 129.11 (3d, ArCH), 137.47 (s, C-1 of Bn ring); *m/z* (FAB$^+$) 279 [(M+H)$^+$, 100%].

7.7.2 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**125**)

To a mixture of **126** (200mg, 0.72mmol), 3Å molecular sieves (200mg) and 1.0M sodium cyanoborohydride in THF (10cm^3) was slowly added a solution of 1.0M hydrochloric acid in ether (9cm^3). TLC (chloroform/acetone 9:1) after 5min indicated consumption of starting material (R_f 0.79) and appearance of product (R_f 0.40). The reaction mixture was diluted with DCM (60cm^3) and filtered. The filtrate was washed

with water (50cm^3) and saturated aqueous $NaHCO_3$ solution (50cm^3). The organic layer was dried ($MgSO_4$), filtered and concentrated to leave a clear yellow residue that was subjected to flash chromatography (eluent chloroform/acetone 9:1), to give the title compound (137mg, 68%).

mp 45–47°C (hexane); $[\alpha]_D^{17}$ +2.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.31, 1.48 (6H, 2s, 2 × isopropylidene CH_3), 3.68 (1H, br s, D_2O exch., OH-3), 3.86–3.96 (2H, m, H-5$_A$, H-5$_B$), 4.23–4.26 (2H, m, H-3, H-4), 4.49 (1H, d, *J* 3.7, H-2), 4.57, 4.61 (2H, AB, J_{AB} 11.9, OC*H*$_2$Ar), 5.96 (1H, d, *J* 3.7, H-1), 7.29–7.38 (5H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.15, 26.72 (2q, 2 × isopropylidene CH_3), 68.16 (t, C-5), 74.05 (t, O*C*H$_2$Ar), 76.19 (d, C-3 or C-4), 78.15 (d, C-2), 85.28 (d, C-3 or C-4), 104.83 (d, C-1), 111.58 (s, isopropylidene *C*(CH$_3$)$_2$), 127.87, 128.03, 128.55 (3d, ArCH), 137.14 (s, C-1 of Bn ring).

A sample of **125** (100mg) was converted to its 3-*O*-acetyl ester by stirring in a mixture of acetic anhydride (1cm^3) and pyridine (4cm^3) overnight. Concentration of the mixture yielded the desired product as a clear oil. R_f 0.65 (chloroform/acetone 14:1).

^{1}H NMR (270 MHz; $CDCl_3$) δ_H 1.30, 1.51 (6H, 2s, 2 × isopropylidene CH_3), 2.00 (3H, s, CH_3CO), 3.65 (2H, d, *J* 5.9, H-5$_A$, H5-$_B$), 4.45–4.50 (3H, m, OCH*H*Ar, H-4, H-2), 4.59 (1H, AB, J_{AB} 12.1, OCH*H*Ar), 5.25 (1H, d, *J* 2.9, H-3), 5.91 (1H, d, *J* 3.9, H-1), 7.26–7.34 (5H, m, ArCH).

7.7.3 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-3-ulose (**129**)

A solution of **125** (400mg, 1.43mmol) in DMSO (1.5cm^3) was added dropwise to acetic anhydride (2cm^3) in DMSO (3cm^3). After stirring for 18h the resulting mixture was added dropwise over 30min to stirred saturated aqueous $NaHCO_3$ solution (50cm^3), and then left to stir for a further 1h before being extracted with DCM (3 × 50cm^3). The combined organic layers were dried ($MgSO_4$), filtered and concentrated, to give the crude unstable ullose (286mg, 72%).

A small amount of the residue was subjected to flash chromatography (eluent chloroform/acetone 19:1) to provide a purified sample for NMR analysis.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.43,1.46 (6H, 2s, 2 × isopropylidene CH_3), 3.73 (2H, d, J 2.4, H-5_A, H-5_B), 4.34–4.35 (1H, m, H-2), 4.44–4.46(1H, m, H-4), 4.49, 4.52 (2H, AB, J_{AB} 12.0, OCH_2Ar), 6.13 (1H, d, J 4.4, H-1), 7.24–7.36 (5H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 27.16, 27.60 (2q, 2 × isopropylidene CH_3), 70.06 (t,C-5), 73.72 (t, OCH_2Ar), 76.81 (d, C-4), 79.91 (d, C-2), 103.57 (d, C-1), 114.15 (s, isopropylidene $C(CH_3)_2$), 127.52, 127.87, 128.47 (3d, ArCH), 137.43 (s, C-1 of Bn ring), 210.02 (s, C-3).

7.7.4 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**130**)

To a pre-cooled solution (0□C) of crude **129** (*ca* 400mg) in ethanol (5cm^3) and water (4cm^3) was added sodium borohydride (70mg, 1.85mmol). TLC (chloroform/ acetone 9:1) after 1h indicated loss of starting material (R_f 0.65), and appearance of a new product (R_f 0.55). Water (25cm^3) was added and this mixture extracted with DCM (3 × 25cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The product was purified by flash chromatography (eluent chloroform/acetone 19:1) to yield 1,2-*O*-isopropylidene-5-*O*-benzyl-α-D-ribofuranose as a crystalline solid (199mg, 50% over two steps).

mp 83–85°C (hexane), [lit.[171] 81–83°C]; $[\alpha]_D^{17}$ +40.2 (*c* 1.0, $CHCl_3$), [lit.[171] $[\alpha]_D^{20}$ +31.2 (*c* 1.87, $CHCl_3$)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.35, 1.55 (6H, 2s, 2 × isopropylidene CH_3), 2.52 (1H, d, J 9.8, D_2O exch., OH-3), 3.62, 3.77 (2H, ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.0, H-5_A, H-5_B), 3.76–3.98 (2H, m, H-3, H-4), 4.53 (1H, dd, J 4.4, H-2), 4.59 (1H, s, OCH_2Ar), 5.81 (1H, d, J 3.9, H-1), 7.26–7.34 (5H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 26.36, 26.43 (2q, 2 × isopropylidene CH_3), 68.51 (t, C-5), 71.59 (d, C-3 or C-4), 73.43 (t, OCH_2Ar), 78.29 (d, C-2), 79.65 (d, C-3 or C-4), 103.98 (d, C-1), 112.46 (s, isopropylidene $C(CH_3)_2$), 127.55, 127.59, 128.26 (3d, ArCH), 137.83 (s, C-1 of Bn ring).

7.7.5 Allyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranoside (**131**)

A solution of **65** (5.00g, 12.5mmol) in acetic anhydride (10cm^3) and pyridine (50cm^3) was stirred for 20 h, and then concentrated repeatedly from toluene to yield the diacetylated product as a clear oil in quantitative yield (6.05g).

m/z (FAB$^+$) 507 [(M+Na)$^+$, 4%], 427 (5), 91 (100); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.89, 2.00 (6H, 2s, 2 × CH_3CO), 3.46, 3.50 (2H, ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.5, H-6_A, H-6_B), 3.59 (1H, dd, *J* 10.0, 3.7, H-2), 3.94 (1H, ddd, *J* 10.3, 2.9, H-5), 3.98–4.03 (1H, m, C*H*HCH=CH_2), 4.15–4.20 (1H, m, CH*H*CH=CH_2), 4.44, 4.58 (2H, AB, J_{AB} 12.2, OCH_2Ar), 4.58, 4.62 (2H, AB, J_{AB} 12.5, OCH_2Ar), 4.85 (1H, d, *J* 3.4, H-1), 5.07 (1H, dd, *J* 9.8, H-4), 5.22 (1H, d, *J* 10.3, CH_2CH=C$H_{cis}CH_{trans}$), 5.32 (1H, d, *J* 17.1, CH_2CH=$CH_{cis}CH_{trans}$), 5.45 (1H, dd, *J* 9.8, H-3), 5.87–5.97 (1H, m, CH_2CH=CH_2), 7.26–7.34 (10H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.70, 20.76 (2q, 2 × CH_3CO), 67.89 (t, C-6), 68.34 (d, C-5), 68.56 (t, CH_2CH=CH_2), 69.23 (d, C-4), 72.22 (d, C-3), 72.95, 73.48 (2t, 2 × OCH_2Ar), 76.73 (d, C-2), 95.56 (d, C-1), 118.27 (t, CH_2CH=CH_2), 127.72, 127.91, 127.94, 127.98, 128.35, 128.46 (6d, ArCH), 133.47 (d, CH_2CH=CH_2), 137.62, 137.84 (2s, 2 × C-1 of Bn ring), 169.82, 170.24 (2s, 2 × CH_3CO).

7.7.6 3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranose (**132**)

Palladium chloride (305mg, 1.72mmol) was added to a vigorously stirred solution of **131** (4.16g, 8.60mmol) in methanol (20cm^3) and DCM (20cm^3). Triethylamine (4cm^3) was added after 3h and the solution concentrated *in vacuo*. The residue was resuspended in ethyl acetate (100cm^3) and filtered through a Celite pad. The filtrate was concentrated and the remaining crude product subjected to flash chromatography (eluent ethyl acetate/hexane 3:7) to yield the desired glucopyranose as a white solid (2.99g, 78%).

R_f 0.26 (ethyl acetate/hexane 3:7); mp 71–74°C; $[\alpha]_D^{17}$ +83.0 (*c* 1.1, $CHCl_3$); (Found: C, 64.70; H, 6.37. Calc for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.88, 1.89. 1.90. 1.96 (6H, 4s, 2 × CH_3CO_α, 2 × CH_3CO_β), 3.80 (0.4H, dd, *J* 7.6, 9.5, H-2_β), 3.46–3.49 (2H, m, H-6_A, H-6_B), 3.57 (0.6H, dd, *J* 3.7, 10.0, H-2_α), 3.64 (0.4H, ddd, *J*

4.2, 10.3, H-5$_\beta$), 4.18 (0.6H, ddd, *J* 3.9, 10.2, H-5$_\alpha$), 4.41–4.64 (3.6H, m, OC*H*$_2$Ar), 4.74 (0.4H, d, *J* 7.8, H-1$_\alpha$), 4.85 (0.4H, AB, J_{AB} 11.7, OC*H*$_2$Ar), 4.93 (0.4H, dd, *J* 10.3, H-4$_\beta$), 5.01 (0.6H, dd, *J* 9.8, H-4$_\alpha$), 5.12 (0.4H, dd, *J* 9.3, H-3$_\beta$), 5.25 (0.6H, d, *J* 3.4, H-1$_\alpha$), 5.45 (0.6H, dd, *J* 9.8, H-3$_\alpha$), 7,23–7.33 (10H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.61, 20.65, 20.72, 20.84 (4q, 4 × *C*H$_3$CO), 68.07 (t, C-6,and d), 68.64 (t, C-6), 69.22, 69.38, 72.02, 72.73 (4d), 72.90, 73.43, 73.48 (3t, 3 × OCH$_2$Ar), 73.99 (d), 74.19 (t, OCH$_2$Ar), 76.88, 79.57 (2d, C-2$_{\alpha \text{ and } \beta}$), 90.76, 97.27 (2d, C-1$_{\alpha \text{ and } \beta}$), 127.72, 127.78, 127.89, 127.98, 128.03, 128.33, 128.36, 128.42, 128.51 (9d, ArCH), 137.27, 137.40 (2s, 2 × C-1 of Bn ring), 169.86, 169.91, 170.33, 170.41 (4s, 4 × CH$_3$*C*O); α and β subscripts denote signals arising from α and β-anomers respectively; *m/z* (FAB$^-$) 443 [(M-H)$^-$, 97%], 597 (100).

7.7.7 3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranosyl dimethyl phosphite (**133**)

Bis(methoxy)(diethylamino)phosphine (966mg, 5.86mmol) was added to a mixture of tetrazole (477mg, 6.76mmol) and **132** in DCM (20cm^3). TLC (ethyl acetate/hexane 2:3) after 30min indicated complete conversion to product R_f 0.65. The reaction mixture was partitioned between ether (100cm^3) and water (80cm^3). The ethereal layer was washed with brine (80cm^3), dried ($MgSO_4$), filtered and concentrated. The resulting clear oil was used without further purification.

^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.88, 1.89, 1.90, 2.01 (6H, 4s, 2 × CH$_3$CO$_\alpha$, 2 × CH$_3$CO$_\beta$), 3.47 (0.5H, 0.5 ABX, $^2J_{AB}$ 11.0, $^3J_{AX}$ 3.9, H-6$_{A\beta}$), 3.50–3.59 (8H, m, H-6$_B$, H-6$_{A\alpha}$, H-2$_\beta$, 2 × POCH$_3$), 3.62 (0.5H, dd, *J* 3.2, 10.0, H-2$_\alpha$), 3.68–3.73 (0.5H, m, H-5$_\beta$), 4.14 (0.5H, ddd, *J* 3.9, 10.3, H-5$_\alpha$), 4.42–4.66 (3.5H, m, OCH$_2$Ar), 4.84 (0.5H, AB, J_{AB} 11.7, OCH*H*Ar), 4.98–5.05 (1H, m, H-4$_\beta$, H-1$_\beta$), 5.11 (0.5H, dd, *J* 9.8, H-4$_\alpha$), 5.17 (0.5H, dd, *J* 9.8, H-3$_\beta$), 5.45 (0.5H, dd, *J* 9.3, H-3$_\alpha$), 5.56 (0.5H, dd, $J_{H\text{-}P}$ 8.3, 3.4, H-1$_\alpha$), 7.26–7.34 (10H, m, ArCH); ^{31}P NMR (161.7 MHz; CD_3OD; ^{1}H decoupled) δ_P 138.54 OP$_\beta$(OMe)$_2$, 139.58 OP$_\alpha$(OMe)$_2$.

7.7.8 3',4'-Di-*O*-acetyl-2',5,6'-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**134**)

A mixture of **130** (1.51g, 5.41mmol), **133** (3.62g, 6.76mmol) and 4Å molecular sieves (6.00g) in dioxane ($36cm^3$) and toluene ($12cm^3$) was stirred for 2h, after which time zinc chloride (1.10g, 8.11mmol) and silver perchlorate (3.36g, 16.22mmol) were added. The flask was wrapped in foil and the reaction mixture stirred for 20h whereupon TLC (ethyl acetate/hexane 3:7) indicated loss of donor (R_f 0.55) and acceptor (R_f 0.24) and appearance of product (R_f 0.24). Sodium bicarbonate (3.00g), ethyl acetate ($100cm^3$) and water ($75cm^3$) were added and the mixture stirred for 30min before being filtered through a celite pad. The organic layer of the filtrate was washed with brine ($100cm^3$), dried ($MgSO_4$), filtered and concentrated to a clear oil that was subjected to flash chromatography to yield the title compound (3.08g, 81% based on acceptor).

mp 125–127°C (diisopropyl ether); $[\alpha]_D^{20}$ +101.6 (*c* 1.6, $CHCl_3$); (Found: C, 66.10; H, 6.42. Calc for $C_{39}H_{46}O_{12}$: C, 66.28; H, 6.56%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.36, 1.53 (6H, 2s, 2 × isopropylidene CH_3), 1.88, 2.03 (6H, 2s, CH_3CO), 3.28, 3.34 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.4, H-6'$_A$, H-6'$_B$), 3.61 (1H, dd, *J* 3.7, 10.0, H-2'), 3.72 (1H, ABX, $^2J_{AB}$ 11.5, $^3J_{AX}$ 3.7, H-5$_A$), 3.79–3.84 (2H, m, H-5$_B$, H-5'), 4.16 (1H, dd, *J* 9.3, 4.4, H-3), 4.29–4.32 (2H, m, OCH*H*Ar, H-4), 4.48–4.71 (6H, m, 2.5 × O*CH*$_2$Ar, H-2), 5.10 (1H, dd, *J* 10.0, H-4'), 5.20 (1H, d, *J* 3.9, H-1'), 5.38 (1H, dd, *J* 9.8, H-3'), 5.83 (1H, d, *J* 3.9, H-1), 7.23–7.31 (15H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.68, 20.89 (2q, 2 × *C*H_3CO), 26.66, 26.72 (2q, 2 × isopropylidene CH_3), 67.23 (t, C-6'), 67.78 (t, C-5), 68.49 (d, C-5'), 68.84, (d, C-4'), 71.51 (t, O*C*H$_2$Ar), 72.06 (d, C-3'), 72.71 (d, C-3), 73.39, 73.59 (2t, OCH_2Ar), 75.62 (d, C-2'), 76.21 (d, C-2), 77.56 (d, C-4), 94.31 (d, C-1'), 104.32 (d, C-1), 113.04 (s, $(CH_3)_2C$), 127.58, 127.69, 127.74, 127.80, 127.98, 128.33 (6d, ArCH), 137.49, 137.80, 138.80 (3s, 3 × C-1 of Bn rings), 169.68, 170.23 (2s, 2 × CH_3CO); *m/z* (FAB$^+$) 729 [(M+Na)$^+$, 8%], 91 (100).

7.7.9 3',4'-Di-O-acetyl-2',5,6'-tri-O-benzyl-3-O-α-D-glucopyranosyl-D-ribofuranose (**135**)

134 (672mg, 0.95mmol) Was heated at reflux for 15min in a mixture of acetic acid/water/ethylene glycol (14/6/3, v/v/v, 25cm^3). After cooling, the reaction mixture was slowly poured into saturated aqueous $NaHCO_3$ solution (75cm^3). This aqueous suspension was extracted with DCM (3 x 75cm^3) and the resulting organic layers were combined, dried ($MgSO_4$), filtered and concentrated repeatedly from toluene to give a yellow oil that was subjected to flash chromatography (eluent ethyl acetate/hexane 1:1) yielding the title compound (477mg, 75%).

mp 126–128°C (ether); R_f 0.45 (ethyl acetate/hexane 1:1); $[\alpha]_D^{20}$ +116.5 (*c* 0.2, $CHCl_3$); (Found: C, 64.90; H, 6.34. Calc for $C_{36}H_{42}O_{12}$: C, 64.85; H, 6.35%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.88, 1.89, 1.99, 2.00 (6H, 4s, 4 × 1.5 CH_3CO), 3.27–3.36 (2H, m, H-6'$_A$, H-6'$_B$), 3.44 (0.4H, d, *J* 6.8, D_2O exch., 1-OH$_\beta$), 3.52–3.67 (3H, m, H-5$_A$, H-5$_B$, H-2'), 3.72 (0.4H, d, *J* 6.3, D_2O exch., 2-OH$_\beta$), 3.82 (0.6H, d, *J* 9.3, D_2O exch., 1-OH$_\alpha$), 3.90 (1H, ddd, *J* 10.3, 3.4, H-5'), 4.00–4.02 (0.4H, m, H-4$_\beta$), 4.12–4.17 (1.2H, m, H-2$_\alpha$, H-3$_\alpha$), 4.24–4.29 (1H, m, H-2$_\beta$, H-4$_\alpha$), 4.32–4.72 (6.8H, m, 3 × OC*H*$_2$Ar, H-3$_\beta$, H-1'$_\beta$), 4.98 (0.6H, d, *J* 3.9, H-1'$_\alpha$), 5.01–5.08 (1H, m, H-4'), 5.26–5.31 (1H, m, H-1), 5.33–5.42 (1H, m, H-3'), 7.22–7.38 (15H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.61, 20.87 (2q, *C*H_3CO), 67.36 (t, C-6'$_\alpha$), 67.46 (t, C-6'$_\beta$), 68.67 (d, C-4'), 69.17 (d, C-5'$_\alpha$), 69.22 (d, C-5'$_\beta$), 69.70 (t, C-5$_\alpha$), 70.10 (t, C-5$_\beta$), 70.57 (d, C-2$_\alpha$ or C-3$_\alpha$), 72.44 (d, C-3'$_\beta$), 72.57 (d, C-3'$_\alpha$), 73.46, 73.53, 73.59,74.21 (4t, 4 × O*C*H$_2$Ar), 74.63 (d, C-4$_\beta$), 75.88 (d, C-2'$_\beta$), 76.01 (d, C-2'$_\alpha$), 77.54 (d, C-2$_\alpha$ or C-3$_\alpha$), 78.55 (d, C-3$_\beta$), 79.83, 80.56 (2d, C-2$_\beta$ and C-4$_\alpha$), 96.98 (d, C-1$_\beta$), 97.53 (d, C-1'$_\beta$), 97.86 (d, C-1'$_\alpha$), 102.47 (d, C-1$_\alpha$), 127.54, 127.67, 127.79, 127.82, 127.94, 128.00, 128.27, 128.33, 128.38, 128.51, 128.73, 128.77 (12d, ArCH), 136.69, 136.74, 137.20, 137.42, 137.86 (5s, 5 × C-1 of Bn rings), 169.69, 170.19 (2s, 2 × CH_3*C*O); α and β subscripts denote signals arising from α and β-anomers respectively; *m/z* (FAB$^+$) 689 [(M+Na)$^+$, 12%], 91 (100).

7.7.10 1,2,3',4'-Tetra-*O*-acetyl-2',5,6'-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose (**124**)

135 (635mg, 0.95mmol) Was stirred in a mixture of acetic anhydride ($0.5cm^3$) and pyridine ($5cm^3$) for 20h. The solution was concentrated repeatedly from toluene to give a runny clear oil that was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7) to yield the title compound (587mg, 82%).

mp 105–107°C (ethyl acetate/hexane); R_f 0.29 (ethyl acetate/hexane 3:7); $[\alpha]_D^{20}$ +98.2 (*c* 0.2, $CHCl_3$); (Found: C, 64.00; H, 6.15. Calc for $C_{40}H_{46}O_{14}$: C, 63.98; H, 6.18%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.86, 1.87, 1.93, 1.96 (12H, 4s, CH_3CO), 3.29, 3.36 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.4, H-6'$_A$, H-6'$_B$), 3.56 (1H, *J* 9.8, 3.4, H-2'), 3.63, 3.72 (2H, ABX, $^2J_{AB}$ 11.2, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.9, H-5$_A$, H-5$_B$), 3.88 (1H, ddd, *J* 10.3, 2.9, H-5'), 4.29 (1H, AB, J_{AB} 12.2, OCH*H*Ar), 4.37–4.40 (1H, m, H-4), 4.47–4.57 (4H, m, 2 × OC*H*$_2$Ar), 4.63–4.64 (2H, m, H-3, OCH*H*Ar), 5.03–5.08 (2H, m, H-1', H-4'), 5.33 (1H, d, *J* 4.9, H-2), 5.38 (1H, dd, *J* 9.3, H-3'), 6.12 (1H, s, H-1), 7.23–7.34 (15H, m, ArCH); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.45, 20.56, 20.63, 20.74, 20.83, 20.94, 21.05, 21.16 (8q, 4 × *C*H$_3$CO$_{\alpha \text{ and } \beta}$), 67.52 (t, C-6'), 68.85 (d, C-5'), 69.00, 69.28 (2t, C-5$_{\alpha \text{ and } \beta}$), 71.89, 72.02 (2d, C-3'$_{\alpha \text{ and } \beta}$), 73.02 (d, C-2), 73.17, 73.26, 73.35, 73.41, 73.48, 73.55 (4t and 2d, C-3$_{\alpha \text{ and } \beta}$, O*C*H$_2Ar_{\alpha \text{ and } \beta}$), 76.59, 76.76 (2d, C-2'$_{\alpha \text{ and } \beta}$), 81.27 (d, C-4), 96.28, 96.47 (2d, C-1'$_{\alpha \text{ and } \beta}$), 98.46, 98.50 (d, C-1$_{\alpha \text{ and } \beta}$), 127.32, 127.61, 127.74, 127.91, 128.02, 128.36, 128.46 (7d, ArCH), 137.51, 137.76, 138.11 (3s, 3 × C-1 of Bn rings), 169.35, 169.69, 170.17, 170.28 (4s, 4 × CH_3CO); α and β subscripts denote signals arising from α and β-anomers respectively; *m/z* (FAB$^+$) 750 (M^+, 1%), 91 (100).

7.8 Synthesis of purinophostin

7.8.1 2',3',5'-Tri-*O*-acetyl 9-β-D-ribofuranosidopurine (**142**)

A suspension of purine in hexamethyldisilazane (7.5cm^3) was refluxed for 7 h. After cooling the mixture was diluted with toluene and concentrated, the residual white powder was the repeatedly concentrated from toluene. 1,2,3,5-Tetra-*O*-acetyl-D-ribofuranose (0.53g, 1.67mmol), dichloroethane (10cm^3) and TMSOTf (3 drops) were added and this mixture was refluxed for 16 h. The reaction was quenched with triethylamine (1cm^3) and diluted with DCM (20cm^3). This organic mixture was washed with 10cm^3 each of saturated aqueous $NaHCO_3$ solution and water and then dried ($MgSO_4$), filtered and concentrated. The crude product was purified by flash chromatography (eluent chloroform/methanol 14:1) to yield the title compound as a clear oil (309mg, 49%).

R_f 0.36 (chloroform/methanol 14:1); ^{1}H NMR (270 MHz; $CDCl_3$) δ_H 2.10, 2.13, 2.17 (9H, 3s, 3 × CH_3CO), 4.37–4.49 (3H, m, H-4', H-5'$_A$, H-5'$_B$), 5.70 (1H, dd, *J* 5.0, H-3'), 6.00 (1H, dd, *J* 5.4, H-2'), 6.27 (1H, d, *J* 5.1, H-1'), 8.27 (1H, s, H-2), 9.02 (1H, s, H-8), 9.18 (1H, s, H-6); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.39, 20.54, 20.76 (3s, 3 × CH_3CO), 63.00 (t, C-5'), 70.56 (d, C-3'), 73.06 (d, C-2'), 80.39 (d, C-4'), 86.43 (d, C-1'), 134.71 (s, C-5), 143.71 (d, C-8), 149.21 (d, C-6), 150.93 (s, C-4), 152.94 (d, C-2), 169.38, 169.59, 170.32 (3s, 3 × CH_3CO).

A sample was converted to the known 9-β-D-ribofuranosidopurine (**143**) as follows: Concentrated aqueous ammonia (20 drops) was added to purine-9-β-D-2', 3', 5'-tri-*O*-acetyl ribofuranoside (279mg, 0.74mmol) in methanol (35cm^3) and the resulting solution was stirred in a sealed flask for 4 h. The resulting reaction mixture was concentrated and the remaining oil repeatedly concentrated from methanol. The product was crystallised from hot methanol (129mg, 70%).

mp 175–178°C. [lit.[215] 181–182°C]; ^{1}H NMR (400 MHz; D_2O) δ_H 3.65, 3.73 (2H, ABX, J_{AB} 12.9, J_{AX} 3.9, J_{BX} 2.7, H-5'$_A$, H-5'$_B$), 4.07–4.09 (1H, m, H-4'), 4.25 (1H, dd, *J* 4.3, H-3'), 4.59 (1H, dd, *J* 4.3, H-2'), 5.93 (1H, d, *J* 5.9, H-1'), 8.44, 8.62, 8.78 (3H, 3s, H-2, H-

6, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 61.52 (t, C-5'), 70.60, 73.98, 85.75 (3d, C-2', C-3', C-4'), 88.50 (d, C-1'), 133.63 (s, C-5), 145.75, 147.93, 151.75 (3d, C-2, C-6, C-8), 150.36 (s, C-4); UV (H_2O) λ_{max} 262nm, ε 6 690, pH 7.5.

7.8.2 2',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (**137**)

A suspension of purine (480mg, 4mmol) in a mixture of hexamethyldisilazane ($10cm^3$) and chlorotrimethysilane ($5cm^3$) was refluxed for 20h. The mixture was concentrated in *vacuo* to a white solid to which were added dichloroethane ($7.5cm^3$), **124** (300mg, 0.40mmol) and TMSOTf ($0.8cm^3$, 0.40mmol); this mixture was refluxed for 7h after which time TLC (ethyl acetate/hexane 3:2) indicated loss of starting material (R_f 0.89) and appearance of one major product (R_f 0.30) and one minor product (R_f 0.11). The reaction mixture was quenched with triethylamine ($1cm^3$), diluted with DCM ($20cm^3$) and washed with saturated aqueous $NaHCO_3$ solution ($15cm^3$) and water ($15cm^3$). The resulting organic layer was dried ($MgSO_4$), filtered and concentrated to leave a clear oil which was purified by flash chromatography (eluent ethyl acetate/hexane 7:3, then 4:1, then ethyl acetate) to give the title compound as a clear oil (198mg, 61%).

$[\alpha]_D^{18}$ +73.6 (*c* 1.4, $CHCl_3$); (Found: C, 63.50; H, 5.82; N, 6.87. Calc for $C_{43}H_{46}N_4O_{12}$: C, 63.69; H, 5.72; N, 6.91%); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.88, 1.94, 1.99 (9H, 3s, 3 × CH_3CO), 3.36–3.43 (2H, m, H-6"$_A$, H-6"$_B$), 3.57 (1H, dd, *J* 10.3, 3.4, H-2"), 3.64, 3.73 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 2.9, $^3J_{BX}$ 2.4, H-5'$_A$, H-5'$_B$), 4.00 (1H, ddd, *J* 9.8, 3.7, H-5"), 4.35, 4.62 (2H, AB, J_{AB} 12.0, OCH_2Ar), 4.49–4.60 (5H, m, 2 × OCH_2Ar, H-4'), 4.75 (1H, dd, *J* 4.4, H-3'), 4.98 (1H, d, *J* 3.9, H-1"), 5.04 (1H, dd, *J* 9.8, H-4"), 5.45 (1H, dd, *J* 9.8, H-3"), 5.72 (1H, dd, *J* 5.1, H-2'), 6.44 (1H, d, *J* 4.9, H-1'), 7.24–7.38 (15H, m, ArCH), 8.41, 8.98, 9.15 (3H, 3s, H-2, H-6, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.36, 20.68, 20.87 (3q, 3 × CH_3CO), 67.92 (t, C-6"), 69.02 (d, C-4"), 69.17 (t, C-5'), 69.28 (d, C-5"), 71.76 (d, C-3"), 73.30, 73.52, 73.72 (3t, 3 × OCH_2Ar), 74.49 (d, C-2'), 76.63 (2d, C-2" and C-3'), 82.86 (d, C-4'), 86.08 (d, C-1'), 98.12 (d, C-1"), 127.82, 127.94, 128.00, 128.12, 128.36, 128.47, 128.62 (7d, ArCH), 134.42 (s, C-5), 137.12,

137.40, 137.54 (3s, 3 × C-1 of Bn rings), 143.71 (d, C-8), 148.81 (d, C-6), 151. 11 (s, C-4), 152.80 (d, C-2), 169.71, 170.21 (2s, 3 × CH_3CO); *m/z* (FAB^+) 811 [$(M+H)^+$, 16%], 691 (13), 91 (100).

Further elution gave the minor product (91mg, 28%), a regioisomer of the desired product which could not be unambiguously identified.

$[\alpha]_D^{18}$ +50.0 (*c* 4.0, $CHCl_3$); (Found: M^+, 811.317. Calc for $C_{43}H_{47}N_4O_{12}$ $(M+H)^+$: 811.319); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.98, 2.05, 2.12 (9H, 3s, 3 × CH_3CO), 3.50–3.54 (2H, m, H-6"$_A$, H-6"$_B$), 3.65–3.68 (2H, m, H-2", H-5'$_A$), 3.71 (1H, ABX, $^2J_{AB}$ 11.0, ^3J 1.9, H-5'$_B$), 4.13 (1H, ddd, *J* 10.2, 4.0, H-5"), 4.47 (1H, AB, J_{AB} 11.7, OCH*H*Ar), 4.59–4.75 (7H, m, H-4', H-3', 5 × OC*H*HAr), 4.99 (1H, d, *J* 3.5, H-1"), 5.10 (1H, dd, *J* 9.8, H-4"), 5.36 (1H, dd, *J* 6.0, H-2'), 5.58 (1H, dd, *J* 9.8, H-3"), 6.30 (1H, d, *J* 6.7, H-1'), 7.34–7.48 (15H, m, ArCH), 8.63, 9.25, 9.34 (3H, 3s, H-2, H-6, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.73, 21.13, 21.36 (3q, 3 × *C*H_3CO), 68.60 (t, C-6"), 69.03 (t, C-5'), 69.44 (d, C-4"), 69.91 (d, C-5"), 72.03 (d, C-3"), 73.63, 73.89, 74.04 (3t, 3 × O*C*H_2Ar), 75.59 (d, C-2'), 76.86 (d, C-2"), 77.76 (d, C-3'), 84.06 (d, C-4'), 88.58 (d, C-1'), 99.20 (d, C-1"), 124.24 (s, C-4 or C-5), 127.81, 127.67, 128.10, 128.15, 128.56, 128.33, 128.39, 128.59, 128.64, 128.73, 128. 89 (11d, ArCH), 136.96, 137.51, 137.54 (3s, 3 × C-1 of Bn rings), 141.82, 146.43, 153.81 (3d, C-2, C-6, C-8), 161.34 (s, C-4 or C-5), 170.24, 170.41 (2s, 3 × CH_3CO); UV ($CHCl_3$) λ_{max} 265nm; *m/z* (FAB^+) 811 [$(M+H)^+$, 15%], 91 (100).

7.8.3 2",5',6"-Tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (**138**)

A solution of **137** (480mg, 0.59mmol), concentrated aqueous ammonia ($5cm^3$), and methanol ($25cm^3$) was stirred in a sealed flask for 20h, and then concentrated. The remaining oil was subjected to flash chromatography (eluent ethyl acetate/ethanol 19:1) to give the desired triol (392mg, 97%).

R_f 0.29 (ethyl acetate/ethanol 19:1); $[\alpha]_D^{20}$ +23.5 (*c* 1.0, $CHCl_3$); (Found: C, 64.80; H, 5.93; N, 8.11. Calc for $C_{37}H_{40}N_4O_9$: C, 64.90; H, 5.89; N, 8.18%); ^1H NMR (400 MHz;

$CDCl_3$) δ_H 3.45 (1H, dd, *J* 9.5, 3.6, H-2"), 3.57, 3.62 (2H, ABX, $^2J_{AB}$ 11.0, $^3J_{AX}$ 2.4, $^3J_{BX}$ 2.4, H-5'$_A$, H-5'$_B$), 3.67–3.74 (3H, m, H-4", H-6"$_A$, H-6"$_B$), 3.94 (1H, ddd, *J* 9.3, 4.2, H-5"), 4.08–4.13 (1H, m, H-3"), 4.23 (1H, dd, *J* 5.1, 2.2, H-3'), 4.33 (1H, br.s., D_2O exch., OH), 4.42–4.43 (1H, m, H-4'), 4.46–4.57 (6H, m, 5 × OC*H*HAr, D_2O exch., OH), 4.66–4.73 (3H, m, H-2', OCH*H*Ar, D_2O exch., OH), 4.82 (1H, d, *J* 3.4, H-1"), 6.30 (1H, d, *J* 6.4, H-1'), 7.24–7.38 (15H, m, ArCH), 8.40, 8.89, 9.14 (3H, 3s, H-2, H-6, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 69.39 (t, C-5'), 69.77 (t, C-6"), 70.08 (d, C-4"), 71.87 (d, C-5"), 72.84 (d, C-3"), 73.66, 73.77, 74.18 (3t, 3 × OCH_2Ar), 75.95 (d, C-2'), 79.19 (d, C-2"), 80.58 (d, C-3'), 83.16 (d, C-4'), 87.97 (d, C-1'), 99.72 (d, C-1"), 127.67, 127.82, 127.87, 128.14, 128.42, 128.58, 128.64, 128.73 (8d, ArCH), 134.56 (s, C-5), 136.67, 137.20, 137.76 (3s, 3 × C-1 of Bn rings), 143.93 (d, C-8), 148.90 (d, C-6), 151.21 (s, C-4), 152.28 (d, C-2); *m/z* (FAB^+) 685 [$(M+H)^+$, 65%], 91 (100).

7.8.4 2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (**139**)

Bis(benzyloxy)(diisopropylamino)phosphine (0.34cm^3, 1.02mmol) and tetrazole (107mg, 1.53mmol) were stirred together in DCM (4cm^3) for 30min, the mixture thus obtained was then added to the starting triol (**138**) (116mg, 0.17mmol). After a further 30min TLC (ethyl acetate/hexane 4:1) indicated conversion of starting material to a single trisphosphite product (R_f 0.80). The reaction mixture was then cooled to -78°C and MCPBA (317mg, 1.10mmol) added, after 5min TLC (ethyl acetate/hexane 4:1) indicated the formation of one product R_f 0.26. 10% Aqueous Na_2SO_3 solution (20cm^3) and ethyl acetate (25cm^3) were added and the mixture was allowed to warm to RT. The organic layer was washed with 20cm^3 each of saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (eluent ethyl acetate/toluene 4:1) yielded the fully protected trisphosphate (198mg, 80%).

$[\alpha]_D^{25}$ +17.9 (*c* 1.4, $CHCl_3$); (Found: M^+, 1465.469. Calc for $C_{79}H_{80}O_{18}P_3$ M^+: 1465.468); ^1H NMR (400 MHz; $CDCl_3$) δ_H 3.53–3.72 (5H, m, H-2", H-5'$_A$, H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.84–3.88 (1H, m, H-5"), 4.30–4.80 (14H, H-3', H-4', H-4", 11 × OC*H*HAr),

4.89–5.07 (8H, m, H-3", 7 × OC*H*HAr), 5.34 (1H, d, *J* 3.5, H-1"), 5.56 (1H, m, H-2'), 6.46 (1H, d, *J* 6.4, H-1'), 6.93–7.42 (45H, m, ArCH), 8.20, 8.85, 9.04 (3H, 3s, H-2, H-6, H-8); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 68.75 (t, C-6"), 69.51–70.21 (7t, C-5', 6 × O*C*H_2Ar with C-P coupling), 70.50 (d, C-5"), 71.95, 73.71, 73.99 (3t, 3 × O*C*H_2Ar), 74.03 (d, C-3'), 74.70 (d, C-4" with C-P coupling), 77.18 (d, C-3" with C-P coupling), 78.34 (d, C-2' with C-P coupling), 82.98 (d, C-4'), 85.68 (d, C-1'), 95.70 (d, C-1"), 127.85, 127.92, 127.99, 128.07, 128.18, 128.25, 128.32, 128.33, 128.54, 128.58, 128.69, 128.76, 128.85, 128.92 (14d, ArCH), 134.50 (s, C-4 or C-5), 134.98–136.39 (6s, 6 × C-1 of benzylphospho rings with C-P coupling), 137.38, 137.82, 138.19 (3s, 3 × C-1 of benzyl rings), 144.17, 148.84, 152.80 (3d, C-2, C-6, C-8), 151.51 (s, C-4 or C-5); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P -0.80, -0.68, -0.12 (3s); *m/z* (FAB$^+$) 1465 [(M^+, 4%], 91 (100).

7.8.5 3'-*O*-α-D-Glucopyranosyl-9-β-D-ribofuranosidopurine 2',3",4"-trisphosphate (**140**)

A mixture of **139** (133mg, 0.09mmol) and wet 20% palladium hydroxide on carbon (400mg), in methanol (22cm^3), cyclohexane (10cm^3) and water (2cm^3) was heated at reflux for 17h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst washed with methanol and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion exchange resin column and eluted with a gradient of 0–100% 150mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20°C) gave the desired product as the free acid (33mg, 56%) which was dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion exchange resin to give, after concentration, the sodium salt.

(Found: M^-, 653.029. Calc for $C_{16}H_{24}N_4O_{18}P_3$ $(M-H)^-$: 653.029); ^{1}H NMR (400 MHz; D_2O) δ_H 3.57–3.75 (6H, m, H-2", H-5'$_A$, H-5'$_B$, H-5", H-6"$_A$, H-6"$_B$), 3.96 (1H, ddd, *J* 9.5, H-4"), 4.30 (1H, dd, *J* 7.3, 3.8, H-4'), 4.36 (1H, ddd, *J* 9.2, H-3"), 4.51 (1H, dd, *J* 5.0, 3.8, H-3'), 5.10 (1H, d, *J* 3.8, H-1"), 5.21 (1H, ddd, *J* 9.4, 4.8, H-2'), 6.40 (1H, d, *J* 5.9, H-1');

^{31}P NMR (161.7 MHz; D_2O; ^{1}H decoupled) δ_P 0.22, 0.71, 1.07 (3s); UV (H_2O) λ_{max} 262nm, ε 6 690, pH 7.5; *m/z* (FAB^-) 653 [$(M-H)^-$, 100%], 551 (32).

7.9 Synthesis of imidophostin

7.9.1 12',3",4"-Tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl)-1-β-D-ribofuranosidoimidazole (**145**)

124 (412mg, 0.55mmol) In 1,2-dichloroethane ($8cm^3$) was added to *N*-trimethylsilylimidazole ($0.09cm^3$, 0.60mmol) and TMSOTf ($0.08cm^3$, 0.41mmol) in 1,2-dichloroethane ($4cm^3$). This solution was heated at reflux for 8h after which time TLC (chloroform/acetone 24:1) indicated starting material (R_f 0.58) still remaining. Further quantities of *N*-trimethylsilylimidazole ($0.05cm^3$, 0.34mmol) and TMSOTf ($0.05cm^3$, 0.26mmol) were added and refluxing continued for a further 16h. TLC then indicated loss of starting material and appearance of one major product (R_f 0.34) and one minor product (R_f 0.23 with streaking). The cooled reaction mixture was diluted with DCM ($20cm^3$) and washed with $20cm^3$ each of saturated aqueous $NaHCO_3$ solution and brine. The resulting organic layer was dried ($MgSO_4$), filtered and concentrated and the residue subjected to flash chromatography (eluent chloroform/acetone 39:1) to yield the title compound as a clear oil (238mg, 57%).

$[\alpha]_D^{18}$ +52.0 (*c* 1.6, $CHCl_3$); (Found: M^+, 759.312. Calc for $C_{41}H_{47}N_2O_{12}$ $(M+H)^+$: 759.312); ^1H NMR (400 MHz; $CDCl_3$) δ_H 1.87, 1.93, 1.99 (9H, 3s, 3 × CH_3CO), 3.37–3.57 (4H, m, H-2", H-5'$_A$, H-6"$_A$, H-6"$_B$), 3.62 (1H, ABX, $^2J_{AB}$ 10.8, $^3J_{BX}$ 2.3, H-5'$_B$), 3.98 (1H, ddd, *J* 10.2, 3.7, H-5"), 4.35 (1H, AB, J_{AB} 11.7, OCH*H*Ar), 4.45–4.61 (7H, m, 5 × OCH*H*Ar, H-3', H-4'), 4.90 (1H, d, *J* 3.5, H-1"), 5.01 (1H, dd, *J* 9.8, H-4"), 5.17 (1H, dd, *J* 5.6, H-2'), 5.43 (1H, dd, *J* 9.7, H-3"), 5.89 (1H, dd, *J* 6.2, H-1'), 7.08 (1H, s, H-5), 7.14 (1H, s, H-4), 7.24–7.35 (15H, m, ArCH), 7.82 (1H, s, H-2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.76, 21.09, 21.31 (3q, 3 × *C*H_3CO), 68.30 (t, C-6"), 69.34 (d, C-4"), 69.61 (d, C-5", and t, C-5'), 72.08 (d, C-3"), 73.12, 73.82, 73.94 (3t, 3 × O*C*H_2Ar), 76.41 (d, C-2'), 76.84 (d, C-2"), 77.79 (d, C-3'), 83.17 (d, C-4'), 88.49, (d, C-1'), 98.69 (d, C-1"), 116.84 (d, C-5), 127.94, 128.00, 128.11, 128.17, 128.24, 128.53, 128.66, 128.75 (8d, ArCH), 129.52 (d, C-4), 136.25 (d, C-2), 137.39, 137.51, 137.61 (3s, 3 × C-1 of Bn rings), 169.77, 170.38 (2s, 3 × CH_3*C*O); *m/z* (FAB^+) 759 [$(M+H)^+$, 54%], 691 (15), 181 (15), 91 (100).

Further elution gave the C_2-symmetrical compound 1,3-bis(2',3",4"-tri-*O*-acetyl-2",5',6"-tri-*O*-benzyl-3'-*O*-α-D-glucopyranosyl-β-D-ribofuranosido) imidazolium (triflate?) (**146**) (81mg, 20% based on 0.5 equivalents of starting disaccharide).
$[\alpha]_D^{18}$ +58.0 (*c* 2.2, $CHCl_3$); (Found: M^+, 1449.582. Calc for $C_{79}H_{89}N_2O_{24}$ M^+: 1449.580); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 1.98, 1.93, 1.97 (18H, 3s, 6 × CH_3CO), 3.86–3.43 (4H, m, 2 × H-6"$_A$, 2 × H-6"$_B$), 3.53, (2H, dd, *J* 10.0, 3.5, 2 × H-2"), 3.59–3.61 (2H, m, 2 × H-5'$_A$), 3.72–3.77 (2H, m, 2 × H-5'$_B$), 3.92 (2H, ddd, *J* 6.2, 3.8, 2 × H-5"), 4.34 (2H, AB, *J* 12.0, 2 × OCH*H*Ar), 4.48–4.50 (10H, m, 4 × OC*H*$_2$Ar, 2 × H-4'), 4.62–4.65 (4H, m, 2 × OCH*H*Ar, 2 × H-3'), 4.99 (2H, dd, *J* 10.0, 2 × H-4"), 5.34 (2H, dd, *J* 4.3, 2 × H-2'), 5.41 (2H, dd, *J* 9.7, 2 × H-3"), 5.99 (2H, d, *J* 3.2, 2 × H-1'), 7.24–7.34 (30H, m, ArCH), 7.51 (2H, br s, H-4, H-5), 9.37 (1H, br s, H-2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 20.73, 21.09, 21.28 (3q, 6 × CH_3CO), 68.56 (t, 2 × C-6"), 68.78 (t, 2 × C-5'), 69.37 (d, 2 × C-4"), 69.66 (d, 2 × C-5"), 71.88 (d, 2 × C-3"), 73.40, 73.81, 73.90 (3t, 6 × OC*H*$_2$Ar), 75.80 (d, 2 × C-3'), 76.20 (d, 2 × C-2'), 76.77 (d, 2 × C-2"), 84.17 (d, 2 × C-4'), 91.40 (d, 2 × C-1'), 98.09 (d, 2 × C-1"), 120.13 (d, C-4 and C-5), 127.99, 128.07, 128.13, 128.41, 128.53, 128.59, 128.85 (7d, ArCH), 134.06 (d, C-2), 137.13, 137.58, 137.72 (3s, 6 × C-1 of Bn rings), 169.79, 170.27, 170.45 (3s, 6 × CH_3*C*O); *m/z* (FAB^+) 759 [$(M+H)^+$, 54%], 691 (15), 181 (15), 91 (100).

7.9.2 2",5',6"-Tri-O-benzyl-3'-O-α-D-glucopyranosyl)-1-β-D-ribofuranosidoimidazole (**147**)

A solution of **145** (240mg, 0.32mmol), concentrated aqueous ammonia (2cm^3), and methanol (10cm^3) was stirred in a sealed flask for 24h, and then concentrated. The residual oil was subjected to flash chromatography (eluent ethyl acetate/ethanol 14:1) to give the desired triol (192mg, 96%).
R_f 0.33 (chloroform/methanol 14:1); $[\alpha]_D^{18}$ +25.8 (*c* 0.7, $CHCl_3$); (Found: M^+, 633.280. Calc for $C_{35}H_{41}N_2O_9$ $(M+H)^+$: 633.281); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.44–3.56 (4H, m, H-2", H-4", H-5'$_A$, H-5'$_B$), 3.62 (1H, ABX, $^2J_{AB}$ 10.4, $^3J_{AX}$ 5.8, H-6"$_A$), 3.70–3.73 (1H, m, H-6"$_B$), 3.83–3.87 (1H, m, H-5"), 4.02 (1H, dd, *J* 9.2, H-3"), 4.17–4.18 (1H, m, H-3'), 4.25 (1H, m, H-4'), 4.30 (1H, dd, *J* 5.6, H-2'), 4.39–4.52 (7H, m, 2 × OC*H*$_2$Ar, D_2O

exch., 3 × OH), 4.68, 4.81 (2H, AB, *J* 11.7, OC*H*$_2$Ar), 4.87 (1H, d, *J* 2.9, H-1'), 5.49 (1H, d, *J* 5.9, H-1"), 6.97 (1H, s, H-5), 7.07 (1H, s, H-4), 7.22–7.33 (15H, m, ArCH), 7.62 (1H, s, H-2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 69.84 (t, C-6"), 70.12 (t, C-5'), 70.96 (d, C-4"), 72.17 (d, C-5"), 73.81, 73.86, 73.95 (3t, 3 × OCH_2Ar), 76.63 (d, C-2'), 78.97 (d, C-2"), 79.88 (d, C-3'), 83.09 (d, C-4'), 90.97 (d, C-1'), 99.85 (d, C-1"), 116.86 (d, C-5), 127.88, 127.91, 127.95, 128.16, 128.58, 128.56, 128.81, 128.86 (8d, ArCH), 129.28 (d, C-4), 136.56 (d, C-5), 137.46, 137.64, 138.20 (3s, 3 × C-1 of Bn rings); *m/z* (FAB$^+$) 633 [(M+H)$^+$, 100%], 91 (81).

7.9.3 2",5',6"-Tri-*O*-benzyl-2',3",4"-tris(dibenzyloxyphosphoryl)-3'-*O*-α-D-glucopyranosyl)-1-β-D-ribofuranosidoimidazole (**148**)

Imidazolium triflate (115mg, 0.53mmol) and bis(benzyloxy) (diisopropylamino)phosphine (0.18cm^3, 0.53mmol) were added to a solution of **147** (104mg, 0.16mmol) in DCM (3cm^3). TLC after 30 min indicated complete conversion the trisphosphite. The reaction mixture was cooled to -78°C and MCPBA (156mg, 0.54mmol) was added, TLC (chloroform/methanol 24:1) after a further 5min indicated oxidation to the protected trisphosphate (R_f 0.39). 10% Aqueous Na_2SO_3 solution (15cm^3) and ethyl acetate (15cm^3) were added and the mixture was allowed to warm to room temperature. The organic layer was washed with 15cm^3 each of saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (eluent ethyl acetate/toluene 7:3) yielded the fully protected trisphosphate (110mg, 72%).

$[\alpha]_D^{18}$ +14.5 (*c* 2.2, $CHCl_3$); (Found: M$^+$, 1413.455. Calc for $C_{77}H_{80}N_2O_{18}P_3$ (M+H)$^+$: 1413.461); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.45–3.48 (1H, m, H-5'$_A$), 3.55–3.59 (2H, m, H-2", H-5'$_B$), 3.64 (1H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 5.4, H-6"$_A$), 3.70–3.73 (1H, m, H-6"$_B$), 3.81–3.85 (1H, m, H-5"), 4.29–4.79 (14H, m, 11 × × OCH*H*Ar, H-4', H-3', H-4"), 4.89–5.05 (9H, m, 7 × × OCH*H*Ar, H-2', H-3"), 5.31 (1H, d, *J* 3.5, H-1"), 5.80 (1H, d, *J* 6.4, H-1'), 6.99 (1H, s, H-5), 7.01 (1H, s, H-4), 7.04–7.35 (45H, m, ArCH), 7.54 (1H, s, H-2); ^{13}C NMR (100.4 MHz; $CDCl_3$) δ_C 68.80 (t, C-6"), 69.38–70.11 (PO*C*H_2Ar with C-P coupling, C-5'), 70.42 (d, C-5"), 71.66, 73.69, 73.90 (3t, 3 × OCH_2Ar), 74.20 (d, C-3'),

74.68 (d, C-4" with C-P coupling), 78.38 (d, C-3" with C-P coupling), 79.10 (d, C-2' with C-P coupling), 82.62 (d, C-4'), 87.67 (C-1'), 95.62 (d, C-1"), 116.81 (d, C-5), 127.83, 127.86, 127.89, 127.94, 127.96, 128.15, 128.20, 128.24, 128.28, 128.40, 128.47, 128.51, 128.57, 128.63, 128.70, 128.74, 128.85, 128.91 (18d, ArCH), 130.11 (d, C-4), 135.22–136.31 (C-1 of benzylphospho rings with C-P coupling), 136.72 (d,C-5), 137.37, 137.86, 138.13 (3s, 3 × C-1 of Bn ring); ^{31}P NMR (161.7 MHz; $CDCl_3$; ^{1}H decoupled) δ_P -0.27, -0.83, -0.97 (3s); *m/z* (FAB$^+$) 1412 (M^+, 7%), 91 (100).

7.9.4 3'-*O*-α-D-Glucopyranosyl)-1-β-D-ribofuranosidoimidazole 2',3",4"-trisphosphate (**149**)

A mixture of **148** (82mg, 0.06mmol) and wet 20% palladium hydroxide on carbon (250mg) in methanol (11cm^3), cyclohexane (5.5cm^3) and water (1cm^3) was heated at reflux for 10h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed with methanol and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion exchange resin column and eluting with a gradient of 0–100% 150mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20°C) gave the desired product as the free acid (33mg, 94%), which was dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion exchange resin to give, after concentration, the sodium salt.

(Found: M^-, 601.024. Calc for $C_{14}H_{24}N_2O_{18}P_3$ $(M-H)^-$: 601.023); ^{1}H NMR (400 MHz; D_2O) δ_H 3.50–3.68 (6H, m, H-2", H-5", H-5'$_A$, H-5'$_B$, H-6"$_A$, H-6"$_B$), 3.91 (1H, ddd, *J* 9.4, H-4"), 4.22–4.31 (3H, m, H-3', H-3", H-4'), 4.68–4.72 (1H, obscured by HDO peak, H-2'), 5.00 (1H, d, *J* 3.5, H-1"), 5.98 (1H, d, *J* 5.3, H-1'), 7.29 (1H, dd, *J* 1.5, H-5), 7.49 (1H, dd, *J* 1.5, H-4), 8.79 (1H, dd, *J* 1.5, H-2); ^{31}P NMR (161.7 MHz; D_2O; ^{1}H decoupled) δ_P 0.13, 0.58, 0.97 (3s); *m/z* (FAB$^-$) 601 [$(M-H)^-$, 100%].

References

1) Evenas, J.; Malmendal, A.; Forsen, S. *Current Opinion In Chemical Biology* **1998**, *2*, 293-302.

2) Berridge, M.J.; Bootman, M.D.; Lipp, P. *Nature (London)* **1998**, *395*, 645-648.

3) Murphy, C.T.; Poll, C.T.; Westwick, J. *Cell Calcium* **1995**, *18*, 245-251.

4) Clapham, D.E. *Cell* **1995**, *80*, 259-268.

5) McPherson, P.S.; Campbell, K.P. *J.Biol.Chem.* **1993**, *268*, 13765-13768.

6) Imagawa, T.; Smith, J.S.; Coronado, R.; Campbell, K.P. *J.Biol.Chem.* **1987**, *262*, 16636-16643.

7) Palade, P.; Dettbarn, C.; Brunder, D.; Stein, P.; Hals, G. *Journal Of Bioenergetics and Biomembranes* **1989**, *21*, 295-320.

8) McPherson, P.S.; Campbell, K.P. *J.Biol.Chem.* **1993**, *268*, 19785-19790.

9) Rubtsov, A.M.; Batrukova, M.A. *Biochemistry-Moscow* **1997**, *62* , 933-945.

10) Lee, H.C.; Walseth, T.F.; Bratt, G.T.; Hayes, R.N.; Clapper, D.L. *J.Biol.Chem.* **1989**, *264*, 1608-1615.

11) Guse, A.H. *Cell.Signal.* **1999**, *11*, 309-316.

12) Berridge, M.J. *Nature (London)* **1993**, *361*, 315-325.

13) Katan, M. *Biochim.Biophys.Acta* **1998**, *1436*, 5-17.

14) Toker, A.; Cantley, L.C. *Nature (London)* **1997**, *387*, 673-676.

15) Marte, B.; Downward, J. *Trends Biochem.Sci.* **1997**, *22*, 355-358.

16) Wakelam, M.J.O. *Biochim.Biophys.Acta* **1998**, *1436*, 117-126.

17) Khan, A.A.; Steiner, J.P.; Klein, M.G.; Schneider, M.F.; Snyder, S.H. *Science* **1992**, *257*, 815-818.

18) Khan, A.A.; Steiner, J.P.; Snyder, S.H. *Proc.Natl.Acad.Sci.USA* **1992**, *89*, 2849-2853.

19) Sharp, A.H.; Snyder, S.H.; Nigam, S.K. *J.Biol.Chem.* **1992**, *267*, 7444-7449.

20) Kuno, M.; Maeda, N.; Mikoshiba, K. *Biochem.Biophys.Res.Commun.* **1994**, *199*, 1128-1135.

21) Malviya, A.N. *Cell Calcium* **1994**, *16*, 301-313.

22) Joseph, S.K. *Cell.Signal.* **1996**, *8*, 1-7.

23) Patel, S.; Joseph, S.K.; Thomas, A.P. *Cell Calcium* **1999**, *25*, 247-264.

24) Riley, A. M. Inositol phosphates and analogues. 1996. University of Bath. Thesis

25) Newton, C.L.; Mignery, G.A.; Sudhof, T.C. *J.Biol.Chem.* **1994**, *269*, 28613-28619.

26) Wojcikiewicz, R.H.; Luo, S.G. *Mol.Pharmacol.* **1998**, *53*, 656-662.

27) Michelangeli, F.; Mezna, M.; Tovey, S.; Sayers, L.G. *Neuropharmacology* **1995**, *34*, 1111-1122.

28) Taylor, C.W.; Broad, L.M. *Trends In Pharmacological Sciences* **1998**, *19*, 370-375.

29) Krause, K.H.; Simmerman, H.B.; Jones, L.R.; Campbell, K.P. *Biochem.J.* **1990**, *270*, 545-548.

30) Mousli, M.; Bueb, J.L.; Bronner, C.; Rouot, B.; Landry, Y. *Trends In Pharmacological Sciences* **1990**, *11*, 358-362.

31) Taylor, C.W.; Richardson, A. *Pharmacology & Therapeutics* **1991**, *51*, 97-137.

32) Challiss, R.J.; Willcocks, A.L.; Mulloy, B.; Potter, B.L.; Nahorski, S.R. *Biochem.J.* **1991**, *274*, 861-867.

33) Bezprozvanny, I.B.; Ondrias, K.; Kaftan, E.; Stoyanovsky, D.A.; Ehrlich, B.E. *Mol.Biol.Cell* **1993**, *4*, 347-352.

34) Yoshida, Y.; Imai, S. *Jpn.J.Pharmacol.* **1997**, *74*, 125-137.

35) Joseph, S.K.; Ryan, S.V. *J.Biol.Chem.* **1993**, *268*, 23059-23065.

36) Komalavilas, P.; Lincoln, T.M. *J.Biol.Chem.* **1994**, *269*, 8701-8707.

37) Harnick, D.J.; Jayaraman, T.; Ma, Y.S.; Mulieri, P.; Go, L.O.; Marks, A.R. *J.Biol.Chem.* **1995**, *270*, 2833-2840.

38) Clementi, E. *Biochemical Pharmacology* **1998**, *55*, 713-718.

39) Maes, K.; Missiaen, L.; Parys, J.B.; Sienaert, I.; Bultynck, M.; Zizi, M.; de Smet, P.; Casteels, R.; De Smedt, H. *Cell Calcium* **1999**, *25*, 143-152.

40) Cardy, T.A.; Taylor, C.W. *J.Biochem.* **1998**, *334*, 455

41) Patel, S.; Harris, S.A.; Adkins, C.E.; O'Beirne, G.; Taylor, C.W. *Proc.Natl.Acad.Sci.USA* **1997**, *94*, 11623-11627.

42) Cameron, A.M.; Steiner, J.P.; Sabatini, D.M.; Kaplin, A.I.; Walensky, L.D.; Snyder, S.H. *Proc.Natl.Acad.Sci.USA* **1995**, *92*, 1784-1788.

43) Irvine, R.F. *FEBS Lett.* **1990**, *263*, 5-9.

44) Berridge, M.J. *Journal Of Physiology-London* **1997**, *499*, 291-306.

45) Thomas, A.P.; Bird, G.J.; Hajnoczky, G.; Robbgaspers, L.D.; Putney Jr., J.W. *FASEB J.* **1996**, *10*, 1505-1517.

46) Taylor, C.W. *Biochimica Et Biophysica Acta-Molecular And Cell Biology Of Lipids* **1998** , *1436*, 19-33.

47) Iino, M.; Endo, M. *Nature (London)* **1992**, *360*, 76-78.

48) Harlan, J.E.; Hajduk, P.J.; Yoon, H.S.; Fesik, S.W. *Nature (London)* **1994**, *371*, 168-170.

49) Hirata, M.; Kanematsu, T.; Takeuchi, H.; Yagisawa, H. *Jpn.J.Pharmacol.* **1998**, *76*, 255-263.

50) Bottomley, M.J.; Salim, K.; Panayotou, G. *Biochim.Biophys.Acta* **1998**, *1436*, 165-183.

51) Falasca, M.; Logan, S.K.; Lehto, V.P.; Baccante, G.; Lemmon, M.A.; Schlessinger, J. *EMBO J.* **1998**, *17*, 414-422.

52) Hyvönen, M.; Macias, M.J.; Nilges, M.; Oschkinat, H.; Saraste, M.; Wilmanns, M. *EMBO J.* **1995**, *14*, 4676-4685.

53) Ferguson, K.M.; Lemmon, M.A.; Schlessinger, J.; Sigler, P.B. *Cell* **1995**, *83*, 1037-1046.

54) Baraldi, E.; Carugo, K.D.; Hyvönen, M.; Surdo, P.L.; Riley, A.M.; Potter, B.V.L.; O'Brien, R.; Ladbury, J.E.; Saraste, M. *Structure* **1999**, *7*, 449-460.

55) Putney Jr., J.W. *Adv.Pharmacology* **1991**, *22*, 251-269.

56) Treiman, M.; Caspersen, C.; Christensen, S.B. *Trends In Pharmacological Sciences* **1998**, *19*, 131-135.

57) Thomas, D.; Kim, H.Y.; Hanley, M.R. *Vitamins And Hormones-Advances In Research And Applications* **1998**, *54*, 97-119.

58) Putney Jr., J.W.; Mckay, R.R. *Bioessays* **1999**, *21*, 38-46.

59) Berridge, M.J. *Biochem.J.* **1995**, *312*, 1-11.

60) Clapham, D.E. *Nature (London)* **1995**, *375*, 634-635.

61) Parekh, A.B.; Penner, R. *Physiol.Rev.* **1997**, *77*, 901-930.

62) Shuttleworth, T.J. *Cell Calcium* **1999**, *25*, 237-246.

63) Putney Jr., J.W.; Bird, G.St.J. *Endocrine Rev.* **1993**, *14*, 610-631.

64) Jayaraman, T.; Ondriasova, E.; Ondrias, K.; Harnick, D.J.; Marks, A.R. *Proceedings Of The National Academy Of Sciences Of The United States Of America* **1995**, *92*, 6007-6011.

65) Sugawara, H.; Kurosaki, M.; Takata, M.; Kurosaki, T. *EMBO J.* **1997**, *16*, 3078-3088.

66) Parekh, A.B.; Terlau, H.; Stuhmer, W. *Nature (London)* **1993**, *364*, 814-818.

67) Randriamampita, C.; Tsien, R.Y. *Nature (London)* **1993**, *364*, 809-814.

68) Kim, H.Y.; Hanley, M.R. *Molecules And Cells* **1999**, *9*, 326-332.

69) Thomas, D.; Hanley, M.R. *J.Biol.Chem.* **1995**, *270*, 6429-6432.

70) Alvarez, J.; Montero, M.; Garciasancho, J. *Biochem.J.* **1991**, *274*, 193-197.

71) Hoebel, B.G.; Kostner, G.M.; Graier, W.F. *Br.J.Pharmacol.* **1997**, *121*, 1579-1588.

72) Shuttleworth, T.J. *J.Biol.Chem.* **1996**, *271*, 21720-21725.

73) Somasundaram, B.; Norman, J.C.; Mahautsmith, M.P. *Biochem.J.* **1995**, *309*, 725-729.

74) Gregory, R.B.; Barritt, G.J. *Biochem.J.* **1996**, *319*, 755-760.

75) Bird, G.S.; Putney Jr., J.W. *J.Biol.Chem.* **1993**, *268*, 21486-21488.

76) Fasolato, C.; Hoth, M.; Penner, R. *J.Biol.Chem.* **1993**, *268*, 20737-20740.

77) Jaconi, M.E.; Lew, D.P.; Monod, A.; Krause, K.H. *J.Biol.Chem.* **1993**, *268*, 26075-26078.

78) Berven, L.A.; Hughes, B.P.; Barritt, G.J. *Biochem.J.* **1994**, *299*, 399-407.

79) Petersen, C.H.; Berridge, M.J. *Biochem.J.* **1995**, *307*, 663-668.

80) Fernando, K.C.; Gregory, R.B.; Barritt, G.J. *Biochem.J.* **1998**, *330*, 1179-1187.

81) Barritt, G.J. *Biochem.J.* **1999**, *337*, 153-169.

82) Huang, Y.; Putney Jr., J.W. *J.Biol.Chem.* **1998**, *273*, 19554-19559.

83) Parekh, A.B. *J.Biol.Chem.* **1998**, *273*, 14925-14932.

84) Mathes, C.; Fleig, A.; Penner, R. *J.Biol.Chem.* **1998**, *273*, 25020-25030.

85) Putney Jr., J.W. *Cell Calcium* **1997**, *21*, 257-261.

86) Potter, B.V.L.; Lampe, D. *Angew.Chem.Int.Ed.Engl.* **1995**, *34* , 1933-1972.

87) Wilcox, R.A.; Primrose, W.U.; Nahorski, S.R.; Challiss, R.A.J. *Trends In Pharmacological Sciences* **1998**, *19*, 467-475.

88) Jenkins, D.J.; Potter, B.V.L. *J.Chem.Soc.Perkin Trans.1* **1998**, 41-49.

89) Riley, A.M.; Jenkins, D.J.; Potter, B.V.L. *J.Am.Chem.Soc.* **1995**, *117*, 3300-3301.

90) Jenkins, D.J.; Riley, A.M.; Potter, B.V.L. *J.Org.Chem.* **1996**, *61*, 7719-7726.

91) Takanari, I.; Kikuchi, K.; Hirose, K.; Iino, M.; Nagano, T. *Bioorg.Med.Chem.Lett.* **1999**, *9*, 1697-1702.

92) Yoshimura, K.; Watanabe, Y.; Erneux, C.; Hirata, M. *Cell Signalling* **1999**, *11*, 117-125.

93) Gawler, D.J.; Potter, B.V.L.; Gigg, R.; Nahorski, S.R. *Biochem.J.* **1991**, *276*, 163-167.

94) Safrany, S.T.; Wilcox, R.A.; Liu, C.; Dubreuil, D.; Potter, B.V.L.; Nahorski, S.R. *Mol.Pharmacol.* **1993**, *43*, 499-503.

95) Mills, S.J.; Riley, A.M.; Murphy, C.T.; Bullock, A.J.; Westwick, J.; Potter, B.V.L. *Bioorg.Med.Chem.Lett.* **1995**, *5*, 203-208.

96) Kozikowski, A.P.; Fauq, A.H.; Wilcox, R.A.; Challiss, R.A.J.; Nahorski, S.R. *J.Med.Chem.* **1994**, *37*, 868-872.

97) Fauq, A.H.; Zaidi, J.H.; Wilcox, R.A.; Varvel, G.; Nahorski, S.R.; Kozikowski, A.P.; Erneux, C. *Tetrahedron Lett.* **1996**, *37*, 1917-1920.

98) Wilcox, R.A.; Fauq, A.H.; Kozikowski, A.P.; Nahorski, S.R. *FEBS Lett.* **1997**, *402*, 241-245.

99) Mezna, M.; Michelangeli, F. *J.Biol.Chem.* **1996**, *271*, 31818-31823.

100) Gafni, J.; Munsch, J.A.; Lam, T.H.; Catlin, M.C.; Costa, L.G.; Molinski, T.F.; Pessah, I.N. *Neuron* **1997**, *19*, 723-733.

101) Maruyama, T.; Kanaji, T.; Nakade, S.; Kanno, T.; Mikoshiba, K. *J.Biochem.* **1997**, *122*, 498-505.

102) Matsuki, N.; Tateishi, K.; Takeuchi, H.; Yagisawa, H.; Kanematsu, T.; Oishi, M.; Hirata, M. *Biochem.Biophys.Res.Commun.* **1999**, *260*, 42-47.

103) Riley, A.M.; Mahon, M.F.; Potter, B.V.L. *Angew.Chem.Int.Ed.Engl.* **1997**, *36*, 1472-1474.

104) Watanabe, Y.; Mitani, M.; Ozaki, S. *Chem.Lett.* **1987**, *1987* , 123-126.

105) Sato, K.; Sakuma, S.; Muramatsu, S.; Bokura, M. *Chem.Lett.* **1991**, *1991*, 1473-1474.

106) Poirot, E.; Bourdon, H.; Chrétien, F.; Chapleur, Y.; Berthon, B.; Hilly, M.; Mauger, J.-P.; Guillon, G. *Bioorg.Med.Chem.Lett.* **1995**, *5*, 569-572.

107) Jenkins, D.J.; Dubreuil, D.; Potter, B.V.L. *J.Chem.Soc.Perkin Trans.1* **1996**, 1365-1372.

108) Cleophax, J.; Dubreuil, D.; Gero, S.D.; Loupy, A.; Vieira de Almeida, M.; Da Silva, A.D.; Vass, G.; Bischoff, E.; Perzborn, E.; Hecker, G.; Lockhoff, O. *Bioorg.Med.Chem.Lett.* **1995**, *5*, 831-834.

109) Dubreuil, D.; Cleophax, J.; de Almeida, M.V.; Verre-Sebrié, C.; Pipelier, M.; Vass, G.; Gero, S.D. *Tetrahedron* **1999**, *55*, 7573-7582.

110) de Almeida, M.V.; Dubreuil, D.; Cleophax, J.; Verre-Sebrié, C.; Pipelier, M.; Prestat, G.; Vass, G.; Gero, S.D. *Tetrahedron* **1999**, *55*, 7251-7270.

111) Riley, A.M.; Payne, R.; Potter, B.V.L. *J.Med.Chem.* **1994**, *37*, 3918-3927.

112) Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J.Antibiot.* **1993**, *46*, 1643-1647.

113) Hirota, J.; Michikawa, T.; Miyawaki, A.; Takahashi, M.; Tanzawa, K.; Okura, I.; Furuichi, T.; Mikoshiba, K. *FEBS Lett.* **1995**, *368*, 248-252.

114) Marchant, J.S.; Parker, I. *Biochem.J.* **1998**, *334*, 505-509.

115) Hartzell, H.C.; Machaca, K.; Hirayama, Y. *Mol.Pharmacol.* **1997**, *51*, 683-692.

116) Huang, Y.; Takahashi, M.; Tanzawa, K.; Putney Jr., J.W. *J.Biol.Chem.* **1998**, *273*, 31815-31821.

117) Machaca, K.; Hartzell, H.C. *J.Biol.Chem.* **1999**, *274*, 4824-4831.

118) DeLisle, S.; Marksberry, E.W.; Bonnett, C.; Jenkins, D.J.; Potter, B.V.L.; Takahashi, M.; Tanzawa, K. *J.Biol.Chem.* **1997**, *272*, 9956-9961.

119) Gregory, R.B.; Wilcox, R.A.; Berven, L.A.; van Straten, N.C.R.; van der Marel, G.A.; van Boom, J.H.; Barritt, G.J. *Biochem.J.* **1999**, *341*, 401-408.

120) Bird, G.S.; Takahashi, M.; Tanzawa, K.; Putney Jr., J.W. *J.Biol.Chem.* **1999**, *274*, 20643-20649.

121) Sato, Y.; Miyazaki, S.; Shikano, T.; Mitsuhashi, N.; Takeuchi, H.; Mikoshiba, K.; Kuwabara, Y. *Biology of reproduction* **1999**, *58*, 867-873.

122) Takahashi, S.; Kinoshita, T.; Takahashi, M. *J.Antibiot.* **1994**, *47*, 95-100.

123) Takaku, H.; Kamaike, K. *Chem.Lett.* **1982**, 189-192.

124) McLaughlin, L.W.; Piel, N.; Hellmann, T. *Synth.Commun.* **1985**, 322-323.

125) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. *J.Am.Chem.Soc.* **1963**, *85*, 3821-3827.

126) Fukuzawa, A.; Sato, H.; Masamune, T. *Tetrahedron Lett.* **1987**, *28*, 4303-4306.

127) Mori, S.; Ohno, T.; Harada, H.; Aoyama, T.; Shioiri, T. *Tetrahedron* **1991**, *47*, 5051-5070.

128) Iversen, T.; Bundle, D.R. *J.Chem.Soc.Chem.Commun.* **1981**, *23*, 1240-1241.

129) Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Kaneko, M. *Tetrahedron Lett.* **1995**, *36*, 5037-5040.

130) Smith, M.; Rammler, D.H.; Goldberg, I.H.; Khorana, H.G. *J.Am.Chem.Soc.* **1962**, *84*, 430-440.

131) Edge, M.D.; Jones, A.S. *J.Chem.Soc.(C)* **1971**, 1933-1939.

132) Michelson, A.M.; Todd, A.R. *J.Chem.Soc.* **1949**, 2476-2486.

133) Chládek, S.; Smrt, J. *Collection Czechoslov.Chem.Commun.* **1963**, *28*, 1301-1308.

134) Montgomery, J.A.; Shortnancy, A.T.; Thomas, H.J. *J.Med.Chem.* **1974**, *17*, 1197-1207.

135) Morley, C.G.D.; Hogencamp, H.P.C. *Arch.Biochem.Biophys.* **1968**, *123*, 207-208.

136) Bakthavachalam, V.; Lin, L.-G.; Cherian, X.M.; Czarnik, W. *Carbohydr.Res.* **1987**, *170*, 124-135.

137) Baggett, N.; Foster, A.B.; Webber, J.M. *Chenistry and Industry* **1965**, 136-137.

138) Hall, D.M. *Carbohydr.Res.* **1980**, *86*, 158-160.

139) Johansson, R.; Samuelsson, B. *Journal Of The Chemical Society-Perkin Transactions 1* **1984**, 2371-2374.

140) Horton, D.; Weckerle, W. *Carbohydr.Res.* **1975**, *44*, 227-240.

141) Jenkins, D.J.; Marwood, R.D.; Potter, B.V.L. *Chem.Commun.* **1997**, 449-450.

142) Hörndler, C.; Pfleiderer, W. *Helv.Chim.Acta* **1996**, *79*, 718-726.

143) Ti, G.S.; Gaffney, B.L.; Jones, R.A. *J.Am.Chem.Soc.* **1982**, *104*, 1316-1319.

144) Pankiewicz, K.W.; Krzeminski, J.; Ciszewski, L.A.; Ren, W.-Y.; Watanabe, K.A. *J.Org.Chem.* **1992**, *57*, 559

145) Greene, T.W.; Wuts, P.G.M.*Protective Groups in Organic Synthesis*; John Wiley & Sons: New York, Chichester, Brisbane, Toronto, Singapore, 1999;

146) Takano, S.; Akiyama, M.; Sato, S.; Ogasawara, K. *Chem.Lett.* **1983**, 1593-1596.

147) Mikami, T.; Asano, H.; Mitsunobu, O. *Chem.Lett.* **1987**, 2033-2036.

148) Uwaydah, I.M.; May, E.L.; Harris, L.S. *J.Med.Chem.* **1977**, *20*, 1374-1377.

149) Hashimoto, S.; Honda, T.; Yanagiya, Y.; Nakajima, M.; Ikegami, S. *Journal Of Synthetic Organic Chemistry Japan* **1995**, *53*, 620-632.

150) Corey, E.J.; Wu, Y.-J. *J.Am.Chem.Soc.* **1993**, *115*, 8871-8872.

151) Watanabe, Y.; Nakamoto, C.; Yamamoto, T.; Shoichiro, O. *Tetrahedron* **1994**, *50*, 6523-6536.

152) Watanabe, Y.; Nakamoto, C.; Ozaki, S. *Synlett* **1993**, 115-116.

153) Watanabe, Y.; Yamamoto, T.; Okazaki, T. *Tetrahedron* **1997**, *53*, 903-918.

154) Schene, H.; Waldmann, H. *Eur.J.Org.Chem.* **1998**, 1227-1230.

155) Gryaznov, S.M.; Letsinger, R.L. *J.Am.Chem.Soc.* **1991**, *113*, 5876-5877.

156) Gryaznov, S.M.; Letsinger, R.L. *Nucleic Acids Res.* **1992**, *20*, 1879-1882.

157) Hayakawa, Y.; Kataoka, M. *J.Am.Chem.Soc.* **1998**, *120*, 12395-12401.

158) Hanessian, S.; Liak, T.J.; Vanasse, B. *Synthesis* **1981**, 396-397.

159) Takahashi, M.; Tanzawa, K.; Takahashi, S. *J.Biol.Chem.* **1994**, *269*, 369-372.

160) van Straten, N.C.R.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron* **1997**, *53*, 6509-6522.

161) Guedat, P.; Schlewer, G.; Krempp, E.; Riley, A.M.; Potter, B.V.L.; Spiess, B. *J.Chem.Soc.Chem.Commun.* **1997**, 625-626.

162) Liu, C.; Davis, R.J.; Nahorski, S.R.; Ballereau, S.; Spiess, B.; Potter, B.V.L. *J.Med.Chem.* **1999**, *42*, 1991-1998.

163) Riley, A.M.; Guedat, P.; Schlewer, G.; Spiess, B.; Potter, B.L. *J.Org.Chem.* **1998**, *63*, 295-305.

164) Riley, A.M.; Murphy, C.T.; Lindley, C.J.; Westwick, J.; Potter, B.V.L. *Bioorg.Med.Chem.Lett.* **1996**, *6*, 2197-2200.

165) Wilcox, R.A.; Challiss, R.A.J.; Traynor, J.R.; Fauq, A.H.; Ognyanov, V.I.; Kozikowski, A.P.; Nahorski, S.R. *J.Biol.Chem.* **1994**, *269*, 26815-26821.

166) Li, W.; Llopis, J.; Whitney, M.; Zlokarnik, G.; Tsien, R.Y. *Nature (London)* **1998**, *392*, 936-941.

167) Hirata, M.; Watanabe, Y.; Ishimatsu, T.; Ikebe, T.; Kimura, Y.; Yamaguchi, K.; Ozaki, S.; Koga, T. *J.Biol.Chem.* **1989**, *264*, 20303-20308.

168) Murphy, C.T.; Riley, A.M.; Lindley, C.J.; Jenkins, D.J.; Westwick, J.; Potter, B.V.L. *Mol.Pharmacol.* **1997**, *52*, 741-748.

169) Jenkins, D.J.; Potter, B.V.L. *J.Chem.Soc.Chem.Commun.* **1995**, 1169-1170.

170) Jenkins, D.J.; Potter, B.V.L. *Carbohydr.Res.* **1996**, *287*, 169-182.

171) Desai, T.; Gigg, J.; Gigg, R. *Carbohydr.Res.* **1996**, *280*, 209-221.

172) Wilcox, R.A.; Erneux, C.; Primrose, W.U.; Gigg, R.; Nahorski, S.R. *Biochem.Soc.Trans.* **1995**, *23*, S 420-S 420

173) Wilcox, R.A.; Erneux, C.; Primrose, W.U.; Gigg, R.; Nahorski, S.R. *Mol.Pharmacol.* **1995**, *47*, 1204-1211.

174) Moitessier, N.; Christian, A.L.; Chapleur, Y.; Humeau, C. *Tetrahedron Lett.* **1995**, 8023-8026.

175) Gigg, J.; Gigg, R. *J.Chem.Soc.Chem.Commun.* **1966**, 82-86.

176) Schmidt, R.R.; Michel, J. *Tetrahedron Lett.* **1984**, *25*, 821-824.

177) Wegmann, B.; Schmidt, R.R. *Journal Of Carbohydrate Chemistry* **1987**, *6*, 357-375.

178) Chandrasekhar, S.; Sumithra, G.; Yadav, J.S. *Tetrahedron Lett.* **1996**, *37*, 1645-1646.

179) Briggs, A.P. *J.Biol.Chem.* **1922**, *53*, 13-16.

180) Marchant, J.S.; Beecroft, M.D.; Riley, A.M.; Jenkins, D.J.; Marwood, R.D.; Taylor, C.W.; Potter, B.V.L. *Biochemistry* **1997**, *36*, 12780-12790.

181) Demchenko, A.; Stauch, T.; Boons, G.-J. *Synlett* **1997**, 818-820.

182) Vaino, A.R.; Szarek, W.A. *Synlett* **1995**, 1157-1158.

183) Onoda, T.; Shirai, R.; Iwasaki, S. *Tetrahedron Lett.* **1997**, *38*, 1443-1446.

184) Yan, L.; Kahne, D. *Synlett* **1995**, 523-524.

185) Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. *J.Org.Chem.* **1998**, *63*, 8815-8824.

186) Tatani, K.; Shuto, S.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 5065-5068.

187) Riley, A.M.; Potter, B.V.L. *Tetrahedron Lett.* **1999**, *40*, 2213-2216.

188) de Kort, M.; Valentijn, G.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron Lett.* **1997**, *38*, 7629-7632.

189) Katayama, E.; Funahashi, H.; Michikawa, T.; Shiraishi, T.; Ikemoto, T.; Iino, M.; Hirosawa, K.; Mikoshiba, K. *EMBO J.* **1996**, *15*, 4844-4851.

190) Murphy, C.T.; Bullock, A.J.; Lindley, C.J.; Mills, S.J.; Riley, A.M.; Potter, B.V.L.; Westwick, J. *Mol.Pharmacol.* **1996**, *50*, 1223-1230.

191) Lampe, D.; Potter, B.V.L. *Tetrahedron Lett.* **1993**, *34*, 2365-2368.

192) Safrany, S.T.; Wilcox, R.A.; Liu, C.; Potter, B.V.L.; Nahorski, S.R. *European Journal Of Pharmacology-Molecular Pharmacology Section* **1992**, *226*, 265-272.

193) Safrany, S.T.; Wojcikiewicz, R.J.H.; Strupish, J.; Nahorski, S.R.; Dubreuil, D.; Cleophax, J.; Gero, S.D.; Potter, B.V.L. *FEBS Lett.* **1991**, *278*, 252-256.

194) Kozikowski, A.P.; Ognyanov, V.I.; Fauq, A.H.; Nahorski, S.R.; Wilcox, R.A. *J.Am.Chem.Soc.* **1993**, *115*, 4429-4434.

195) Douglas, N.L.; Ley, S.V.; Lücking, U.; Warriner, S.L. *J.Chem.Soc.Perkin Trans.1* **1998**, 51-65.

196) Jiang, L.; Chan, T.-H. *J.Org.Chem.* **1998**, *63*, 6035-6038.

197) Cai, J.; Davison, B.E.; Ganellin, R.C.; Thaisrivongs, S. *Tetrahedron Lett.* **1995**, *36*, 6535-6536.

198) Liao, W.; Lu, D. *Carbohydr.Res.* **1996**, *296*, 171-182.

199) Moravcova, J.; Capkova, J.; Stanek, J. *Carbohydr.Res.* **1994**, *263*, 61-66.

200) Chen, T.Q.; Greenberg, M.M. *Tetrahedron Lett.* **1998**, *39*, 1103-1106.

201) Garegg, P.J.; Hultberg, H.; Wallin, S. *Carbohydr.Res.* **1982**, *108*, 97-101.

202) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem.Ber.* **1981**, *114*, 1234-1255.

203) Vorbrüggen, H.; Höfle, G. *Chem.Ber.* **1981**, *114*, 1256-1268.

204) Nair, V.; Richardson, S.G. *J.Org.Chem.* **1980**, *45*, 3969-3974.

205) Al Mourabit, A.; Beckmann, M.; Poupat, A.; Ahond, A.; Potier, P. *Tetrahedron: Asymmetry* **1996**, *7*, 3455-3464.

206) James, M.N.G.; Matsushima, M. *Acta Cryst.* **1972**, *B29*, 838-846.

207) Miles, D.W.; Revankar, G.R.; Robins, R.K. *J.Phys.Chem.* **1983**, *87*, 2444-2450.

208) Mitra, C.; Saran, A.; Govil, G. *Ind.J.Chem.* **1978**, *16B*, 132-136.

209) van Straten, N.C.R.; Kriek, N.M.A.J.; Cziria, Z.A.C.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron* **1997**, *53*, 6539-6554.

210) van Straten, N.C.R.; van der Marel, G.A.; van Boom, J.H. *Tetrahedron* **1997**, *53*, 6523-6538.

211) Beecroft, M.D.; Marchant, J.S.; Riley, A.M.; van Straten, N.C.R.; van der Marel, G.A.; van Boom, J.H.; Potter, B.V.L.; Taylor, C.W. *Mol.Pharmacol.* **1999**, *1*, 109-117.

212) Hotoda, H.; Murayama, K.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Keneko, M. *Biochemistry* **1999**, *38*, 9234-9241.

213) Still, W.C.; Kahn, M.; Mitra, A. *J.Org.Chem.* **1978**, *43*, 2923-2925.

214) Talley, E.A.; Vale, M.D.; Yanovsky, E. *J.Am.Chem.Soc.* **1945**, *67*, 2037-2039.

215) Nair, V.; Chamberlin, S.D. *Synth.Commun.* **1984**, 401-403.